

BIOANALYTICAL SENSORS, BIOCHIPS AND NANOBIOTECHNOLOGIES

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Deuxième Atelier France-Israël
Second France-Israel Workshop
with an international participation

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L'avenir passe par les micro et nano biotechnologies

AUTRANS. Le centre Maeva a accueilli, cette semaine, le 2^e atelier franco-israélien sur les technologies et les avancées conceptuelles majeures en micro et nano biotechnologies

Une centaine de chercheurs israéliens, américains et européens se sont retrouvés, cette semaine, au centre Maeva d'Autrans pour évoquer les technologies et les avancées conceptuelles majeures dans les domaines des micro et nano biotechnologies. Que ce soit dans ceux de la médecine, de l'environnement ou de l'alimentation de nombreux tests, à partir des biocapteurs, sont conduits actuellement : prélèvement, analyse en laboratoire et vérification des résultats. Ceux-ci ont l'avantage d'offrir un résultat immédiat au moyen d'une manipulation simple et à la portée de tous. L'atout majeur de ces petits appareils : détecter, reconnaître et informer en temps réel des composés indésirables sur les éléments biologiques. De multiples applications existent d'ores et déjà dans de nombreux domaines. Ainsi, pour l'environnement, il est possible au moyen d'un biocapteur trempé dans le liquide d'obtenir aussitôt une analyse de la qualité de l'eau. L'appareil détecte et avertit de la présence de produits indésirables, même à faible concentration. On peut donc tout à fait imaginer que lors de pollutions de grande importance, le biocapteur, grâce à la rapidité de ses résultats, permette de réagir plus rapidement qu'actuellement. Il en va de même pour la qualité de l'air.

Côté alimentation, les biocapteurs pourraient bien aider la France à faire que la listeria ne menace plus nos fromages en permettant aux producteurs de tester eux-mêmes leurs produits. Ils peuvent au jour le jour trier immédiatement le bon grain de l'ivraie et éviter de manière systématique de mettre sur le marché des produits dangereux pour la santé... Autre champ d'application, celui de la médecine. Là aussi, la recherche fait de grandes avancées. Certains traitements comportant des effets secondaires sur des maladies telles que celle de Parkinson ou d'Alzheimer peuvent être modifiés au moyen d'un biocapteur, également capable de lire et de corriger les données du traitement en fonction du patient. La nanotechnologie, cette science de l'infiniment petit, permet de mettre biopuces et biocapteurs, notamment, au service de la médecine en régulant à l'intérieur du corps dosage et suivi de médicaments dans certaines maladies comme le diabète. Inconvénient : les phénomènes de rejet restent extrêmement sensibles. De l'infiniment petit à l'infiniment grand, il y a un espace pas aussi grand qu'on pourrait l'envisager. Un jour, relativement proche, les fusées seront obsolètes. Les nano technologies, les biocapteurs et autres biopuces les



Serge Cosnier, directeur de la recherche au C.N.R.S. et Robert Marks de l'université Ben Gourion, coorganisateur de cette rencontre.

remplaceront. Ils ne pèseront rien, accompliront un travail extrêmement précis et d'une discrétion absolue dans l'espace. Ils pourront donc aller partout. Si les nano-biotechnologies sont en train de faire leurs preuves et ont, pour la plupart, été validées, reste que le marché français est lent à se mettre en route et les contrats rares. Il n'empêche que la collaboration avec Israël s'avère enrichissante dans la confrontation des divers organes de recherche et fructueuse lors des rencontres.

Claire ANTONOWICZ ■

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Deuxième Atelier France - Israël sur les Capteurs Bioanalytiques, les Biopuces et les Nanobiotechnologies

Organisé sous les auspices des :

Ministère des Affaires Etrangères, France



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- Ville de Grenoble, France

Deuxième Atelier France - Israël sur les Capteurs Bioanalytiques, les Biopuces et les Nanobiotechnologies

Motivations

A l'initiative des Docteurs Serge Cosnier (université Joseph Fourier) et Robert Marks (université Ben Gurion), un congrès « France-Israël Bi-National workshop on Biosensors and Biochips » s'est déroulé à Omer du 27 au 28 Octobre 1998. Le programme de ce congrès était axé sur la conception et la réalisation de micro-systèmes analytiques ou de microcapteurs incluant des molécules biologiques (enzymes, ADN, anticorps, oligonucléotides) et leur exploitation en analyse pour l'environnement, les processus industriels ou le biomédical. Une complémentarité scientifique évidente est apparue entre la France et Israël qui se concrétise actuellement par l'établissement de collaborations dans ce domaine particulièrement sensible.

Dans ce contexte prometteur, il a semblé extrêmement judicieux d'organiser un nouveau colloque en France afin de consolider et d'étendre ces premières relations binationales. Ainsi un projet d'atelier international France-Israël sur les capteurs bioanalytiques, les biopuces et les nanobiotechnologies a été proposé et accepté par le Ministère israélien des Sciences et des Arts et le Ministère français des Affaires Etrangères via l'Ambassade de France en Israël.

Ce congrès est axé sur les microbiocapteurs, immunocapteurs et la fonctionnalisation biologique de microstructures à visées analytiques. Une session sera particulièrement dévolue à la conception et au développement des biopuces en France et en Israël ainsi qu'aux avancées technologiques afférentes en micro et nano-électroniques. Afin d'assurer une ouverture thématique et une expertise optimale, la participation à ce congrès binational a été étendue au niveau international.

Description du congrès et de ses objectifs

Le principal objectif de cette manifestation consiste en la présentation et la confrontation des technologies émergentes et des avancées conceptuelles majeures dans les domaines des diagnostics bioanalytiques et des micro et nano-biotechnologies afin d'induire des collaborations entre scientifiques relevant de disciplines différentes ainsi que des contacts avec les industriels impliqués dans ces domaines. Il s'agira également d'accueillir des

scientifiques de réputation internationale travaillant dans le domaine des diagnostics bioanalytiques ainsi que de jeunes chercheurs débutant dans ce domaine pluridisciplinaire.

Le congrès sera constitué de conférences plénières sur invitation (7) et d'une sélection de conférences (34) organisées autour de sessions thématiques (7) qui décriront d'une part les principes fondamentaux afférents à chaque thème et d'autre part développeront les nouveaux concepts les plus pertinents dans ce domaine. Par ailleurs, une présentation de communication par affiche sera organisée pendant toute la durée du congrès. De plus, une soirée sera consacrée à des communications orales flash de ces affiches suivies d'une table ronde pour un débat informel. Ces différentes présentations montreront les convergences interdisciplinaires actuellement en cours dans le domaine du diagnostic.

Les différents thèmes concernent les biocapteurs (électrochimiques, optiques, acoustiques, piezoélectriques, magnétiques, thermiques, implantables, basé sur des enzymes, des récepteurs ou des acides nucléiques, les immunocapteurs), les microarrays, les microréacteurs, la bioélectronique, la microfluidique, la micro-optoélectronique, le lab-on-chip, les puces à ADN, les biopuces, la protéinomie, la bio-informatique et les technologies de détection.

Le champ des thèmes à discuter englobera les concepts novateurs mais également les tests en conditions réelles des technologies émergentes, leur commercialisation potentielle et la position de ces nouveaux outils analytiques vis à vis des principales méthodes analytiques en biochimie, en immunologie et en génétique. La contribution d'industriels et de professionnels de la distribution d'instruments analytiques sera vivement encouragée.

Une définition réaliste des champs d'applications visés par les biocapteurs, les biopuces et les nanobiotechnologies dans une large variété de domaines tels que la médecine, les laboratoires d'analyses, l'environnement, l'agriculture, la génomique, la défense et les industries de la nourriture et des boissons, sera entreprise. La présence d'universitaires impliqués dans la recherche fondamentale et/ou appliquée associée à celle d'industriels devrait faciliter le transfert de connaissances entre différents domaines tels que la nano et l'optoélectronique, la chimie, la biologie et la physique.

Une issue spéciale de Talanta, une revue internationale de chimie analytique pure et appliquée, sera réalisée à partir des manuscrits présentés à cet Atelier.

Second France-Israel Workshop on Bioanalytical Sensors, Biochips and Nanobiotechnologies

Appropriateness of the workshop

This workshop in the Grenoble area in France (December 11-17, 2000) is the reciprocal of the First France-Israel workshop on biosensors and biochips, held in Omer, Israel in 27-28 OCTOBER, 1998. It has raised interest in other countries and we have expanded the contents to nanobiotechnologies and have included other countries which are contributors to the fields. The aforementioned fields are expanding fast and therefore such a workshop will help bring together decision makers and developers to create collaborations and allow them to brainstorm for new concepts. In addition, the workshop should help promote collaborations between Israeli and French laboratories or companies.

Meeting description and objectives

Science and medicine promise the possibilities of revolutionary diagnostic and therapeutic techniques. In particular, biosensors are the subject of increasing research efforts and constitute now a major component of mainstream analytical chemistry. Their application areas generally include analytical, environmental and clinical laboratories. The development of assay techniques that have the convenience of solid-phase hybridisation and are rapid, sensitive and readily multiplexed will have a significant impact on diagnostics and genomics. In addition, new tools have become available (like DNA chips) and some technologies have become more popular such as micro and nano-fabricated electroanalytical systems, quartz crystal microbalance monitoring or electrochemical biomolecule immobilization.

In this context, the main objective of the « The Second France-Israel Workshop on Bioanalytical Sensors, Biochips and Nanobiotechnology » is to gather the expertise of scientists from an interdisciplinary background and working at the forefront of analytical diagnostics. The workshop is an interdisciplinary five day event consisting of invited plenary presentations (7) and selected oral contributions (34), which describe relevant and important new concepts and will underpin understanding of the field. In addition, an exhibition of poster presentations and flash communications is expected to foster further interactions.

The aforementioned presentations will pool together state-of-the-art disciplines in the field of diagnostics, which have evolved separately, but are now finding themselves

converging one to another. These are biosensors (including electrochemical, optical, acoustic-piezoelectric, magnetic, thermometric, implantable, continuous, discontinuous, enzyme-based, receptor-based, nucleic acid-based, immunosensors), actuators, microarrays, microreactors, high-throughput screening, bioelectronics, micromechanics, microfluidics, microoptoelectronics, lab-on-a-chip, DNA-chips, protein biochips, proteomics, bioinformatics and detection technologies.

Drug discovery is one of the key areas to benefit from these technologies, particularly relevant given the tremendous push by pharmaceutical and biotechnology companies to streamline research activities and to integrate promising enabling technologies into their discovery pipeline.

The range of topics to be discussed includes innovative concepts, reality check on future enabling technologies, their potential commercialization and position in mainstream analytical bio/chemistry, immunology or genetics.

Application areas are to be found in medicine, analytical laboratories, veterinary, environment, food and beverage industries, agriculture, genomic applications, security pharmaceutical (drug discovery), and defense.

We have invited academics involved not only in basic research but also in entrepreneurship as well as companies developing such items. We hope it will facilitate the transfer of knowledge between different fields such as nanoelectronics, optoelectronics, chemistry, biology and physics.

An initial invitation to publish the workshop proceedings in 'Talanta' has been offered.

Second France-Israel Workshop on Bioanalytical Sensors, Biochips and Nanobiotechnologies

Programme

Monday 11 December

- 17:00 Registration and poster set-up (Centre MAEVA, Autrans)
20:00 Reception and Dinner

Tuesday 12 December

- 9:00 **Greetings**
Prof. Feuerstein, President – *Université Joseph Fourier Grenoble, France*

Session Biomimetic sensors-System models

Prof. I. Willner, <i>The Hebrew University of Jerusalem, Israel</i> – chairman

Keynote lecture :

- 9:30 **Dr. André Dittmar, *INSA de Lyon, France***
Biomedical microsensors : new trends and impact in medicine

10:05 **Coffee Break**

Lectures :

- 10:35 **Dr. Sharon Marx, *IIBR Ness-Ziona, Israel***
Molecular imprinting in thin films sol-gel vs. acrylic polymers
- 11:00 **Dr. Daniel Riveline, *Université Joseph Fourier Grenoble, France***
Controlling the motion of motor proteins : the electric motility assay
- 11:25 **Prof. Mathias Winterhalter, *IPBS CNRS UMR 5089 Toulouse, France***
Membrane channels used to control the enzyme activity
- 11:50 **Prof. Abraham Shanzer, *The Weizmann Insitute of Science Rehovot, Israel***
From surface modification to molecular sensors
- 12:15 **Lunch**

Session Electrochemical transduction of biological events

Dr. G. Bidan, CEA-Grenoble, France – chairman

Keynote lecture :

- 14:00 **Dr. Christian Amatore, Ecole Normale Supérieure Paris, France**
Artificial synapses : detection and analysis of individual exocytotic cellular events
with ultramicroelectrodes

Lectures :

- 14:35 **Prof. Jean-Michel Kauffmann, Université Libre de Bruxelles, Belgique**
Mediated bioelectrocatalysis of horse radish peroxidase
- 14:50 **Prof. Adrian C. Michael, University of Pittsburgh, USA**
Monitoring chemistry in the extracellular space of the brain with bioanalytical sensors
based on carbon fiber microelectrodes
- 15:15 **Prof. Judith Rishpon, Tel-Aviv University, Israel**
Recombinant single chain antibodies in electrochemical detection of pathogens
- 15:50 **Coffee Break**
- 16:20 **Dr Omowunmi A Sadik, State University of New York, USA**
Generic strategy for reversible immunosensors using pulsed electrochemical detection.
- 16:45 **Dr. E. N. K. Wallace, The Queen's College Oxford, UK**
Direct electrochemistry of putidaredoxin and its interactions with cytochrome P450_{cam}

Session Poster

Prof. J. M. Kauffmann, Université Libre de Bruxelles, Belgique - chairman

- 17:20 **Oral presentations (2 transparents, 5 min)**

- 19:00 **Dinner**

Wednesday 13 December

Session Impact of new sensing technologies on biomolecular interface

Prof. L. Blum, Université Claude Bernard Lyon, France- chairman

Keynote lecture :

- 9:00 **Prof. Itamar Willner, The Hebrew University of Jerusalem, Israel**
Amplified electronic transduction of DNA detection processes

Lectures :

- 9:35 **Dr. Olivier Bagel, Ecole Polytechnique Fédérale de Lausanne, Suisse**
Reverse iontophoresis for subcutaneous glucose detection
- 10:00 **Prof. Ovadia Lev, The Hebrew University of Jerusalem, Israel**
Scanning capillary microscopy for spatial bioanalytical and analytical applications
- 10:25 **Coffee Break**
- 10:55 **Dr. Ziv Reich, Weismann Institute Rehovot, Israel**
Single complexes to single molecules using AC SPM and capacitance-based motion detection
- 11:20 **Prof. Eliora Ron, Tel Aviv University, Israel**
Electrochemical on-line and *in-situ* monitoring of gene expression

Session Optical and Acoustic-piezoelectric sensors

Prof. A. Shanzer, Ben-Gurion University of the Negev Beer-Sheva, Israel- chairman

Lectures :

- 11:45 **Dr. Marcus Textor, ETH Zurich, Suisse**
Modification of metal oxide surfaces for optical biosensor. Applications based on assembled monolayers of functionalized alkane phosphates and poly(L-lysine)-g-poly(ethylene glycol).
- 12:10 **Lunch**
- 14:00 **Snow racket**
- 16:30 **Tea Time (Vin chaud et Thé)**
- Lectures :**
- 17:00 **Dr. Anne Glover, University of Aberdeen, Scotland UK**
Rapid bioluminescence-based biosensor analysis of environmental samples.

- 17:25 **Dr. Hubert Perrot**, *Université Pierre et Marie Curie Paris, France*
Study of antigen/antibody interactions through piezoelectric measurements
- 17:50 **Dr. Thierry Livache**, *CEA Grenoble, France*
Surface plasmon resonance detection of DNA hybridization on polypyrrole support
- 18:15 **Dr. Robert Marks**, *Ben-Gurion University of the Negev Beer-Sheva, Israel*
RecA-lux optic-fiber biosensor to genotoxics
- 18:40 **POSTER**
- 20:00 **Banquet**

Thursday 14 December

Session Biochips and DNA Sensors

Prof. L. Shamansky, *California State University Riverside, USA*- chairman

Keynote lecture :

- 10:00 **Prof. Marco Mascini**, *Epidemiologia e Chimica Analitica Ambientale Firenze, Italy*
Electrochemical DNA biosensors

Lectures :

- 10:35 **Dr. Gérard Bidan**, *CEA-Grenoble, France*
Contributions of electrochemistry to DNA-biochips
- 11:00 **Dr. Jean-Pierre Cloarec**, *Ecole Centrale de Lyon, France*
Contribution of chemists to DNA chips implementation
- 11:25 **Dr. Claudia Preininger**, *Austria Research Centers Seibersdorf, Austria*
New approaches to oligonucleotide immobilization for application in chip technology
- 11:50 **Dr. Françoise Vinet**, *CEA-Grenoble, France*
A new strategy for in situ synthesis of oligonucleotide arrays for DNA chip technology
- 12:15 **Lunch**

Session Biomolecule immobilization on transducers

Dr O. A. Sadik *State University of New York, USA*- chairman

Keynote lecture :

- 14:00 **Prof. Jean-Louis Marty**, *Université de Perpignan, France*
Enzyme sensors based on NAD-dependent dehydrogenases

Lectures :

- 14:35 **Prof. Lisa Shamansky**, *California State University San Bernardino, USA*
Immobilization and detection of DNA molecules on microfluidic devices
- 15:00 **Dr. Serge Cosnier**, *Université Joseph Fourier Grenoble, France*
Controlled complex architectures of biosensors based on electropolymerized films
- 15:25 **Prof. Roger Leblanc**, *University of Miami, USA*
Molecular interactions between acetylcholinesterase and organophosphorus compounds in Langmuir and Langmuir-Blodgett films
- 15:50 **Dr. Daniel Mandler**, *The Hebrew University of Jerusalem, Israel*
Immobilization of organic and biomolecules with high spatial resolution using the scanning electrochemical microscope (SECM)
- 16:15 **Coffee Break**
- 16:45 **Dr. Sabine Szunerits**, *Université Joseph Fourier Grenoble, France*
A rapid and easy procedure of biosensor fabrication by micro-encapsulation of enzyme in hydrophilic synthetic Latex films.
- 17:10 **Dr. Edward Cummings**, *University of Ulster, Northern Ireland, UK*
Amperometric screen printed enzyme electrodes for the detection of phenolic compounds
- 17:35 **Dr. Avi Halperin**, *CEA Grenoble, France*
Design of DNA chips : Theoretical issues.
- 18:00 **POSTER**
- 20:00 **Dinner**

Friday 15 December

Session Luminescence-fluorescence-bioluminescence
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Dr. R. Marks , <i>Ben-Gurion University of the Negev Beer-Sheva, Israel</i> - chairman

Keynote lecture :

- 9:00 **Prof. Shimshon Belkin**, *The Hebrew University of Jerusalem, Israel*
Probing the environment with genetically engineered microbial sensor cells

Lectures :

- 9:35 **Prof. Loïc Blum**, *Université Claude Bernard Lyon, France*
Electrochemiluminescence as a tool for the development of electro-optical biosensors

10:00 **Dr. Christa Baumstark-Khan**, *German Aerospace Centre Cologne, Germany*
The microscale application of the fluorometric analysis of DNA unwinding (μ -FADU)
as a mammalian genotoxicity assay

10:25 **Coffee Break**

Session Nanobiotechnologies

Prof. J. Cooper *University of Glasgow, Scotland UK*- chairman

Keynote lecture :

10:55 **Dr. Levi A. Gheber**, *Ben-Gurion University of the Negev Beer-Sheva, Israel*
Clusters of membrane proteins revealed with near-field scanning optical microscopy
(NSOM)

12:00 **Lunch**

Lecture :

14:05 **Dr Gianfranco Gilardi**, *Imperial College of Science London, U.K.*
Engineering novel redox assemblies for nanobiotechnology

14:30 **Dr. Nicole Jaffrezic Renault**, *Ecole Centrale de Lyon, France*
Enhancement of the ionic response of field effect transducers using porous silicon

14:55 **Dr. Mario Feingold**, *Ben-Gurion University of the Negev Beer-Sheva, Israel*
Single molecule study of DNA formamide interaction

15:20 **Prof. Jonathan Cooper**, *University of Glasgow, Scotland UK*
Picolitres, zeptomoles and millikelvin : progress towards biological nanosensors

15:45 **Coffee Break**

Saturday 16 December

Informal discussion

KEYNOTES

SESSIONS :

1-Biomimetic sensors-System models	15
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BIOMEDICAL MICROSENSORS : NEW TRENDS AND IMPACT ON MEDECINE

A. Dittmar, G. Delhomme

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At the beginning of this new millennium, the world is becoming more and more health conscious and health care is evolving in many ways :

The patient's needs are changing...

- Non invasive, mini invasive... painless methods of diagnostics and treatments
- Home care, ambulatory methods, short stays in the hospital, telemedicine
- Enhancement of rehabilitation engineering
- Information and involvement of the patient in their treatment

Health policy is changing...

- Limited health care budgets, managed care, ...
- Improvement and control of healthcare quality
- Changes in society (way of life, ageing...)
- The possibilities of individual and long distance mobility
- The "information society" resulting of synergies between computers, teletransmissions and sensors.
- Prevention rather treatment; prediction rather than response

In the face of these changes, progress in science and technology offer, for the first time, many new possibilities and solutions, bringing intelligence, speed, miniaturization, sophistication, new materials... at low cost. In this new landscape of health and technology, **microtechnologies are a key factor** in attempts to meet the changing needs in medicine and biology:

The small size allows:

Minimally invasive surgery.... multimicrosensors and micro implant devices....
r e d u c e d p a i n
and tissue damage.... ambulatory instrumentation.... Reduced reagent and power consumption.... **intelligent and disposable devices**.... and more...

In addition, the physics of the micro scale can provide new functionality often do to **phenomena that exist only at the microscale**. These phenomena are providing new tools and capabilities for medicine and biology.

At the micro or nanoscale, the frontier between physics, chemistry, optics, materials, ... often becomes blurry requiring a multidisciplinary approach.

The synergy (and the conflict) between fast and large evolution & multidisciplinary problems **requires a new behaviour in research, industry and medicine**. Exchange, cooperation and networking **will be the keys to the future success of this field**.

ARTIFICIAL SYNAPSES: DETECTION AND ANALYSIS OF INDIVIDUAL EXOCYTOTIC CELLULAR EVENTS WITH ULTRAMICROELECTRODES.

Christian AMATORE

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Living cells exchange information through the emission of chemical messengers. The importance of such messengers has been widely recognized by biologists. However what is less understood is how these chemical messengers are released by the cell in its outer-cytoplasmic fluids. This difficulty is easily understood when one becomes aware that most of these releases occur in the atto- or femtomole ranges which prevents the use of classical analytical methods. We wish to show here that ultramicroelectrodes may prove extremely useful for monitoring such events.¹

In this lecture we will be concerned by exocytosis of neurotransmitters.¹⁻⁵ The target cells are chromaffin cells which are located above kidneys. These cells produce the initial adrenaline burst which induces fast body reactions ; they are used in neurosciences as standard models for synaptic exocytosis by catecholaminergic neurons. Prior to exocytosis, adrenaline is contained in highly concentrated solutions into a gel matrix packed into small vesicles dispersed in the cytoplasm near the cell membrane. Stimulation of the cell by divalent ions induces the fusion of the vesicles membranes with that of the cell and hence the release of the intravesicular content into the outer-cytoplasmic region. Electrochemical data permit to describe the whole process of exocytosis with a precision that has never been achieved before by patch-clamp techniques. This enables to investigate kinetically these events and conclude upon the physicochemical origin of the individual factors which govern vesicular release. Based on this analysis, one may propose the first explanation for the biologically known relationship between size of an exocytotic vesicle and type of release : mostly through pore-release for vesicles less than 25-30 nm radius and containing less than 10,000-20,000 neurotransmitter molecules ; mostly through full-fusion release for larger vesicles.

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AMPLIFIED ELECTRONIC TRANSDUCTION OF DNA DETECTION PROCESSES

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Amplified electronic transduction of DNA or RNA sensing processes is accomplished by the coupling of a biotinylated oligonucleotide to the probe/target-DNA recognition pair, or by the polymerase-induced or reverse transcriptase-induced replication of genomic DNA or RNA in the presence of biotinylated-dCTP, followed by the binding of an enzyme-avidin conjugate to the assembly. The linked enzyme stimulates the precipitation of an insoluble product on the electronic transducers (i.e. electrodes or piezoelectric crystals). The biocatalytic formation of the precipitate amplifies the DNA (or RNA) recognition events. The sensing processes reveal unprecedented sensitivity and specificity. The genome DNA of mp13 or the viral RNA of vesicular stomatitis virus were sensed by this method.

Single point mutations in DNA are sensed by the polymerase-induced attachment of biotinylated bases complementary to the mutation sites, followed by the association of an enzyme-avidin conjugate that precipitates an insoluble product on the sensing interface. The method was applied for the analysis of chromosomal DNA that carries the Tay-Sachs genetic disorder. By a sequence of analyses, the normal gene could be differentiated from the homocytotic and heterocytotic genes. The analyses were performed with no pre-PCR amplification of the analyzed samples.

A different approach for the amplified transduction of DNA sensing events involves the assembly of DNA-crosslinked nanoparticle arrays. The photonic, electronic and photoelectrochemical transduction of the DNA recognition events will be addressed.

DNA sensing and recognition events as well as single-base mutations are detected at the molecular level using atomic force microscopy (AFM) and measuring the adhesive forces between the target sensing site associated with the AFM-tip and appropriate-functionalized surface. The adhesive forces between a peptide nucleic acid probe-functionalized AFM tip with an undecane thiol monolayer associated on an Au surface, decreases upon the recognition of the target DNA. The different aspects related to the sensing of DNA from the nanometric molecular level to the macroscopic level of chromosomal gene detection will be addressed.

DNA ELECTROCHEMICAL BIOSENSORS

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The determination of low-molecular weight compounds with affinity for DNA can be measured by their effect on the oxidation signal of the guanine peak of calf thymus immobilised on the electrode sensor and investigated by chronopotentiometric analysis. The DNA biosensor is able to detect known intercalating and groove binding compounds. Detection limits of 0.3, 0.2, 10 mg l^{-1} were obtained for daunomycin, polychlorinated biphenyls (PCBs) and aflatoxin B1, respectively. Applicability to river water samples and to waste water for toxicity evaluation is demonstrated.

Moreover we developed new procedures for the detection of hybridization by coupling DNA electrochemical sensors with Polymerase Chain Reaction.

The electrochemical sensor was realized by immobilizing simple stranded oligonucleotides onto graphite screen-printed electrodes by adsorption at controlled potential. The hybridization reaction occurred on the electrode surface was evidenced by chronopotentiometric stripping analysis using daunomycin as indicator.

With the use of two different probes, it was possible to investigate both the positions in which the apoE polymorphism in human blood samples takes place and thus to distinguish different genotypes. The procedure was validated with a reference method based on polyacrilamide gel electrophoresis.

ENZYME SENSORS BASED ON NAD⁺-DEPENDENT DEHYDROGENASES

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With more than 250 enzymes, NAD⁺-dependent dehydrogenases appear promising for the detection of a great variety of substrates in agro-food industry. In such sensors the electrochemical detection is based on the oxidation of NADH produced by the enzymatic reaction. The main drawbacks of dehydrogenase-based sensors are related to the necessary addition of the expensive cofactor NAD⁺ in the reaction medium, and to the low oxidising power of NAD⁺ which drives the equilibrium of many dehydrogenase-catalysed reactions to the reactant side.

The first problem can be solved by immobilising a NAD⁺ macromolecular derivative (NAD⁺-PEG or NAD⁺-dextran) together with enzymes in a PVA-SbQ matrix, or by using a chronoamperometric detection method in which the enzymes and the cofactor are simply deposited on the surface of a horizontally-held electrode.

The second drawback can be overcome by several methods :

- coupling the dehydrogenase with a NADH-oxidising enzyme (diaphorase or NADH oxidase), the oxidation of NADH being coupled to the reduction of an electronic acceptor (ferricyanide or hydrogen peroxide), which can be electrochemically reoxidised;

- immobilisation in the electrode material of a properly selected mediator, or electrochemical functionalization of the electrode surface, enabling the direct oxidation of NADH at low potential.

The different aspects of each method are discussed and an application to the detection of wine fermentation products is presented.

PROBING THE ENVIRONMENT WITH GENETICALLY ENGINEERED MICROBIAL SENSOR CELLS

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Bacteria can be genetically engineered to generate an easily quantifiable signal in response to diverse pre-determined chemical stimuli; bacterial strains can be thus "tailored" to detect and report the presence of either highly specific or very broad groups of chemicals. This can be achieved by the fusion of two genetic elements; the first, the promoter, senses the presence of the target compound and activates the second, the reporter. The specificity of the construct is determined by the choice of promoter, while the nature of the signal is dictated by the reporter genes.

Using either bioluminescence or fluorescence as reporter functions, we have constructed a series of whole-cell bacterial sensors which sensitively respond to the presence of:

- Specific compounds such as halogenated organics
- Broader classes of chemicals including DNA-damaging agents and general toxicants
- Other types of environmental conditions such as nutrient depletion or oxidative stress

Examples will be presented for all of these cases, and the biotechnological potential for the application of the general approach will be discussed.

CLUSTERS OF MEMBRANE PROTEINS REVEALED WITH NEAR-FIELD SCANNING OPTICAL MICROSCOPY (NSOM)

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Near-field scanning optical microscopy (NSOM) uses the near-field interaction of light from a sharp fiber-optic probe with a sample of interest to image surfaces at a resolution beyond the diffraction limit of conventional optics. This technique presents an obvious advantage for biological applications, since it offers resolution comparable with electron microscopy, yet without the need for destructive sample preparation. We describe a NSOM specially designed and built with biological applications in mind, including problems associated with imaging soft and corrugated samples in liquid. With the aid of this instrument we have imaged clusters of the MHC-I protein on the plasma membrane of human skin fibroblast, of ~300 nm diameter, and have proposed a computer based model to explain the mechanism leading to their formation.

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MOLECULAR IMPRINTING IN THIN FILMS SOL-GEL VS. ACRYLIC POLYMERS

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Molecular imprinting is a relatively new technique used to create recognition sites in polymeric matrices. The recognition is based on non-covalent interactions between an analyte molecule to functional monomers prior to the polymerization. Polymerization with high degree of crosslinking, and subsequent removal of the analyte, leaves a cavity in the polymer that is complementary both in size and geometry with specifically positioned binding sites. Molecular imprinting has found many uses in the preparation of selective stationary phases for liquid chromatography. The use of molecularly imprinted polymers as recognition elements in biosensors is highly desirable. The polymers are advantageous to antibodies in terms of chemical and physical stability, function in non-aqueous medium, diversity of analytes and cost.

Molecular imprinting toward the β -blocker drug propranolol was accomplished in two different polymer matrices: the hybrid organic-inorganic sol-gel, and the more "classic" matrix for molecular imprinting – the methacrylic acid- ethylene glycol dimethacrylate (MAA-EGDMA) matrix. The polymers were applied as sub-micron films on glass supports. The binding between the imprinted polymer films and propranolol was investigated using methods like radioactive ligand binding, fluorescence and Quartz Crystal Microbalance (QCM).

The molecular imprinting in the sol-gel was found to be superior to imprinting in acrylic polymers in terms of binding efficiency, reproducibility, non-specific binding, stability and ease of preparation. The binding and recognition properties of the imprinted polymer towards propranolol were found to possess high specificity and selectivity. The binding constant between propranolol and the sol-gel film was found to be $K_D=80$ nM, which is characteristic to natural antibodies.

CONTROLLING THE MOTION OF MOTOR PROTEINS : THE ELECTRIC MOTILITY ASSAY.

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We have developed a novel technique which allows to direct the two dimensional motion of actin filaments on a myosin coated sheet using a weak electric field parallel to the plane of motion. The filament velocity can be increased or decreased, and even reversed, as a function of orientation and strength of the field. PMMA (poly(methylmethacrylate)) gratings, which act as rails for actin, allow for the first time to explore three quadrants of the force velocity diagram. We discuss effective friction, duty ratio and stall force at different myosin densities. A discontinuity in the velocity force relationship suggests the existence of a dynamical phase transition. These experiments show how to direct the translational motion of actin filaments over long distances in non-biological environments via properly engineered tracks, and how to regulate their speed non-invasively.

MEMBRANE CHANNELS USED TO CONTROL THE ENZYME ACTIVITY

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OmpF is a general diffusion porin from the outer cell wall of the Gram-negative bacteria *Escherichia coli*. It has the advantage of being well characterised, its 3-D crystal structure is known, mutagenesis allows to modify specific parts on a regular basis, the protein is stable and available in large quantity. This suggests to use it for biotechnological application. For example, we used it as a sensor to quantify the translocation of antibiotic molecules on a single molecular level. Another application is to encapsulate single enzyme molecules into small lipid or polymeric nanometersized container. The turnover of the encapsulated enzyme is limited by the permeability of the substrate across the vesicle wall. The presence of OmpF allows to control the permeability through the channel properties. We demonstrate this on the activity of encapsulated beta-lactamase in absence and in presence of reconstituted OmpF channels. In a similar system we encapsulated acetylcholineesterase, an enzyme being blocked by insecticides and with possible application as a biosensor.

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MEDIATED BIOELECTROCATALYSIS OF HORSE RADISH PEROXIDASE

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The enzyme Horse Radish Peroxidase (HRP) as well as Glucose Oxidase and Alkaline Phosphatase are likely the biological components most often investigated in biosensor design. Employed as such, or exploited as enzyme label in immunosensors, the HRP offers remarkable catalytical properties in addition to a fairly good stability. Numerous electrochemical biosensor configurations have been described in the literature with the HRP immobilized physically or chemically onto the electrode surface. Of particular interest is the fact that HRP possesses its redox active prosthetic relatively accessible for direct electron transfer to occur at the electrode solution interface. Biosensor configurations with direct detection of HRP activity allow detection of hydrogen peroxide down to μM concentration. However for improved efficiency of electron transfer between HRP and the electrode, the use of a redox mediator is preferable. This allows considerable signal amplification for highly sensitive determination of peroxides (nM) and HRP (pM). Efforts are focused on judicious selection of the redox mediator and on the most adequate immobilization of HRP and the mediator for high efficiency.

Following our recent studies on HRP based carbon composite electrodes for phenothiazine compounds of pharmacological interest and hydrogen peroxide determination [1,2], it appeared that the molecule phenothiazine represented a particularly efficient redox mediator for HRP. The present study was concerned with the investigation of the kinetic study of the homogeneous bioelectrocatalysis between HRP and several mediators in a comparative manner. Cyclic voltammetry and chronoamperometry experiments were implemented at a solid carbon paste electrode (sCPE) and the data analysed following the general expressions for catalytic currents in the presence of redox mediators with reversible or irreversible electron transfer characteristics. The $\text{Fe(III)/Fe(II), H}_2\text{O}_2$ system was first studied as a catalytical model. The second order rate constant k'_t was found to be $70 \text{ M}^{-1} \text{ s}^{-1}$. General expression of the kinetic behaviors were established and perfect fitting between experimental data and mathematical model was observed.

Subsequently several redox mediators were investigated in the presence of the $\text{HRP/H}_2\text{O}_2$ system in acetate buffer solution. The mediators investigated were: 1,4-p benzoquinone, 1,1'-dimethylferrocene, phenol, 4-methylcatechol, promazine and promethazine. Cyclic voltammetry at the sCPE allowed the identification of the redox mechanism at the electrode solution interface. HRP peroxidation of the mediator in solution was found to mimic the electrochemical behavior. Due to the instability of some redox mediators in solution in the presence $\text{HRP/H}_2\text{O}_2$, extrapolation of the chronoamperometric curves to time 0 allowed calculation of mediator concentration and the determination of the second order constant between HRP and the mediator. This was clearly observed in the case of the phenothiazine cation radical of promazine and promethazine. Phenol redox mediation was not related to the phenol/phenoxyl radical couple but actually identified as being due to the new redox couples (likely o-quinones) generated by the enzymatic system. Second order rate constants were: $7.3 \cdot 10^5 \text{ M}^{-1} \text{ s}^{-1}$ (p.benzoquinone), $9.6 \cdot 10^4 \text{ M}^{-1} \text{ s}^{-1}$ (dimethylferrocene), $1 \cdot 10^4 \text{ M}^{-1} \text{ s}^{-1}$ (phenol), $7 \cdot 10^2 \text{ M}^{-1} \text{ s}^{-1}$ (4-methylcatechol), $2 \cdot 10^6 \text{ M}^{-1} \text{ s}^{-1}$ (promazine) and $6.5 \cdot 10^6 \text{ M}^{-1} \text{ s}^{-1}$ (promethazine).

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RECOMBINANT SINGLE CHAIN ANTIBODIES IN ELECTROCHEMICAL DETECTION OF PATHOGENS

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Recombinant antibodies provide an emerging strategy in the development of new immunosensors. In particular, single-chain antibodies (scFvs) can be isolated and expressed in bacterial systems that also allow their *in vitro* manipulation at the gene level. Antibody-phage display libraries have been increasingly used in the generation of specific binders of proteins that recognize membrane receptors, continuous epitopes, and soluble proteins. As such, scFvs may be used for ligand capture/immobilization and for ligand detection – two necessary components of an immunosensor. We have isolated from a phage display library several enzyme-specific, as well as whole bacteria-specific, scFvs.

In this report, we present the development of electrochemical immunosensors that are based on specific scFvs. The scFvs is immobilized on screen-printed carbon electrodes and then used to capture and quantify its specific target antigen. Several examples will be presented:

(a) The first example shows an immunosensor for β -galactosidase that is based on the ability of an anti β -galactosidase scFv to capture the enzyme. The scFv was isolated from a phage library and expressed in *E. coli* as a fusion protein containing a cellulose-binding domain (CBD). The anti β -galactosidase scFv-CBD fusion protein was immobilized onto a cellulose filter, placed on a screen print carbon electrode, and the enzyme activity was measured by lactose injection.

(b) In a second example, we used two isolated antibody fragments (ScFv), prepared against the bacterium *Listeria monocytogenes*, to detect the microorganism in a sandwich immunoassay. One antibody was isolated from a phage library and expressed in a CBD-containing fusion protein, and the other was an identical antibody fused to alkaline phosphatase.

(c) A third example was aimed at identifying and quantifying the enzymatic activity of an enzyme having both catalase and peroxidase activity, *MtKatG*, which is expressed by *Mycobacterium tuberculosis*.

GENERIC STRATEGY FOR REVERSIBLE IMMUNOSENSORS USING PULSED ELECTROCHEMICAL DETECTION

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The uses of biomolecular components (such as enzymes DNA and antibodies) have been advocated in the development of new sensing technologies. Various electrochemical sensing technologies based on enzymes have been described, the most famous being the glucose sensor. The utilization of antibodies in electrochemical sensing technologies has not been so successful. Apparently, there is no convenient, sensitive and selective electrical signal generation mechanism available. A basic, unresolved question in electroimmunochemistry is how to overcome the difficulties associated with the generation of rapid, sensitive and reversible antibody-antigen (Ab-Ag) interactions. We have shown that the use of antibody immobilized onto conducting polymeric matrices, together with pulsed-electrochemical detection (PED) technique enabled selective molecular recognition. A further fascinating phenomenon observed with this system was the capacity to control Ab-Ag interaction, thus making reversibility possible, and rendering the immunological sensors reusable. PED utilizes the advantage of the change in capacitance at the surface of an organic, conducting polymer membrane to generate reversible analytical signals. The selectivity of the resulting sensor can be manipulated through an appropriate choice of the sensing chemistries and the applied potential. When coupled with flow injection analysis, PED also provides a basis for kinetic studies of Ab-Ag reactions through the in-situ measurements of the association and dissociation processes. This technique has been demonstrated for a variety of analytical applications, including clinical, industrial and environmental analytes. Such sensors resulted in detection limits in the low parts-per-billion range, with selectivities of less than 2%. The principle and analytical applications of pulsed accelerated immunosensor technologies will be presented.

DIRECT ELECTROCHEMISTRY OF PUTIDAREDOXIN AND ITS INTERACTIONS WITH CYTOCHROME P450_{CAM}

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Over the last twenty years there has been much interest in cytochrome P450 enzymes due to the wide range of reactions catalysed. The electrochemically driven turnover is of great interest because of its potential both in the development of stereo-specific synthesis and in the production of novel biosensors. This driving force has led to a plethora of approaches in an attempt to obtain successful electron transfer; however to date the turnover rates are disappointingly low. One of the acknowledged problems in this area is the difficulty of transferring the second electron to the system. It has been proposed that this can be overcome by using the natural redox partner of the P450 to transfer electrons from the electrode surface to the cytochrome. We will present results which show that we can successfully achieve the direct electrochemistry of the natural redox partner of P450_{CAM}, putidaredoxin. The potential of this observed redox couple is in good agreement with the biochemical data, thermodynamically enabling electron transfer to P450_{CAM}. We will show preliminary results, where P450 addition indicates that electron transfer to the cytochrome can be successfully achieved. Finally we will compare this approach with a second methodology involving the site directed mutagenesis P450_{CAM}.

REVERSE IONTOPHORESIS FOR THE GLUCOSE MONITORING

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Iontophoresis is the application of a small electric current density ($< 0.5 \text{ mA.cm}^{-2}$) to the skin in order to facilitate the transdermal passage of molecules. An interesting feature of iontophoresis is its symmetry: the same electric field facilitates the entry into the body of exogenous molecules (therapy aspect of the project) and favours the extraction of endogenous molecules (reverse iontophoresis for the diagnosis aspect). The latter allows the noninvasive clinical monitoring of medical interest molecules, such as glucose.

A polymer μ -structure (achieved by UV laser photoablation), with an internal volume of a few nanoliters, that combines electroosmotic pumping and electrochemical detection is particularly well adapted to analyse the small volumes of subcutaneous fluid obtained by this technique (5 to 10 $\mu\text{L.cm}^{-2}.\text{h}^{-1}$). The device is composed of an iontophoretic unit and an analytical μ -channel. The iontophoretic unit consists of a screen-printed cathode in a collection reservoir and a screen-printed anode in contact with the skin. The subcutaneous fluid extracted is dragged to the μ -channel, which serves as the reaction chamber where the analyte is detected.

This concept has been applied to glucose monitoring. Although glucose is not charged, iontophoresis increases dramatically the passage of this polar sugar across the skin. In vivo measurements have been achieved and will be discussed. Finally, a second generation of μ structures which allow to deliver drugs as well as to extract endogenous compounds will be presented.

SCANNING CAPILLARY MICROSCOPY/MASS SPECTROMETRY FOR MAPPING SPATIAL ELECTROCHEMICAL AND ACTIVITY

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A new technique for microscopic imaging of electrochemically and biochemically active surfaces is introduced. The technique combines concepts of probe microscopy and advances in mass spectrometry. The technique is based on a miniature electrochemical flow cell scanner. A liquid feed stream containing a redox component is introduced to the vicinity of the examined location through the annulus of a coaxial capillary set. The incoming reagent interacts with the target location and the generated product stream is transferred through the inner capillary to an Electrospray Interfaced Mass Spectrometer, ESI/MS. Thus, a multicomponent, potential dependent image of the products' distribution versus the location on the electrode is generated. The use of the technique is demonstrated by scanning the electrochemical heterogeneity of model electrodes and by enzymatic reactions.

ELECTROCHEMICAL ON-LINE AND *IN-SITU* MONITORING OF GENE EXPRESSION

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On-line and *in-situ* monitoring of gene expression can be performed by using an electrochemical biosensor-based technology. The biosensing is based on the concept of gene fusion - molecular fusion of reporter genes to promoters of interest. In our case the reporter genes code for enzymes that can be assayed electrochemically. The electrochemical measurements are performed using an array of disposable electrochemical cells, based on screen print electrodes, that allow on-line monitoring of several genes simultaneously. In principle, it is possible to monitor the expression of any gene, given the ability to genetically clone its promoter. We demonstrated the use of the technology with bacteria, yeast and human cell cultures, for the monitoring of several physiological processes, including entrance of cultures to stationary phase, response to anaerobic conditions and to viral infection. In addition, the technology was used for the development of biosensors for monitoring environmental pollutants - using promoters that respond to heavy metals or to organic pollutants we could monitor *in situ* and on line the presence of pollutants such as cadmium and toluene. The sensitivity of the biosensors is such that the presence of ppb concentration of the pollutants can be determine in environmental samples without any pretreatment of the sample. This technology for monitoring gene activity is also potentially useful for a wide variety of biotechnological applications, such as the on-line monitoring of fermentation processes, where the exact state of the culture at any given time, and especially the concentration of the required fermentation product, are important parameters.

MODIFICATION OF METAL OXIDE SURFACES FOR OPTICAL BIOSENSOR APPLICATIONS BASED ON ASSEMBLED MONOLAYERS OF FUNCTIONALIZED ALKANE PHOSPHATES AND POLY(L-LYSINE)-G-POLY(ETHYLENE GLYCOL)

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Long-chain alkane phosphates spontaneously self-assemble out of aqueous solutions onto high refractive index transition metal oxide layers such as tantalum, niobium or titanium oxide. They attach coordinatively through the phosphate head groups to the transition metal cations at the surface and form partially ordered self-assembled monolayers (so-called SAMs) with defined physico-chemical properties such as wettability or surface charges. Highly hydrophobic surfaces can be produced using methyl-terminated dodecylphosphate, medium hydrophilic ones by using hydroxy-terminated alkylphosphates. Such surfaces may subsequently be "passivated" with biotinylated albumin, thus forming a sensor platform characterized by low non-specific binding and the possibility to bind streptavidin and biotinylated recognition units to this surface. The results of bioaffinity sensing assays based on the system alkane phosphate SAM//biotinylated BSA//biotinylated anti-IgG//IgG//cy5-labeled secondary anti-IgG are presented.

A second, novel approach to a versatile optical sensing platform involves the use of poly(L-lysine) grafted with poly(ethylene oxide) (PLL-g-PEG), a polycationic block copolymer that spontaneously assembles as a monolayer at negatively charged metal oxide surfaces such as those formed by silicon, titanium, tantalum or niobium oxide. The interaction with the negatively charged surface is shown to be electrostatic through the terminal amine groups of the poly(L-lysine) side chains charged positively at pH below 9. The surfaces have been characterized *ex situ* using X-ray photoelectron spectroscopy, time-of-flight secondary ion mass spectrometry and reflection-absorption infrared spectroscopy. The planar optical waveguide (grating coupler) technique was used *in situ* both to monitor in real time the assembly process at the metal oxide waveguide surface, as well as to determine the degree of non-specific adsorption when exposed to serum. The degree of protein resistance was found to depend on the PLL-g-PEG coverage, on the grafting ratio between lysine monomer units and PEG side chains, and on the molecular weight of the PEG used. Using optimized polymer architectures, very low values of serum adsorption could be achieved, typically below the detection limit of our optical waveguide instrument (1 ng/cm²). The surfaces remain protein-resistant in flowing buffer solution at least up to 7 days. Functionalized PLL-g-PEG molecules were synthesized with functional groups such as biotin at the terminal position of the PEG side chains. The functionality of these polymer layers on optical waveguide chips was investigated using a model assay with streptavidin binding, followed by the adsorption of biotinylated recognition units and targeting of proteins such as IgG.

Both types of surface functionalization are shown to have an excellent potential for future applications in the area of bioaffinity sensing, based on high refractive index optical waveguide techniques. They can cost-effectively be applied to surfaces by aqueous dipping processes and allow one to tailor the surfaces in terms of very low non-specific adsorption and controlled exposure of specific recognition units. The differences, advantages and disadvantages of the two novel type of biosensor surfaces developed will be discussed.

BIOLUMINESCENT BIOSENSORS AS RAPID INDICATORS OF TOXICITY

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A suite of whole cell biosensors has been constructed by introducing bioluminescence genes into a variety of host organisms ranging from environmentally relevant bacteria to single cell and multicellular eukaryotic hosts. These biosensors are constitutively bioluminescent and their light output is directly related to cell metabolism. Therefore, if the cell is exposed to a sample containing toxic compounds, the bioluminescence will show a corresponding decrease in light output. This decrease is rapid (seconds) and reproducible and reflects the susceptibility of the host cell to the challenge compound. The kinetics of decrease in light varies depending upon the nature of the toxic insult to the cell and this can be exploited to indicate whether the toxicity is organic or inorganic in nature. We can also distinguish between toxicity to prokaryotic systems and toxicity to eukaryotic systems by choosing a particular biosensor. The normal format for the biosensors is in aqueous solution although they can be immobilised onto thin films and have been used successfully in on-line systems.

So far, the biosensors have been used very effectively to diagnose environmental pollution in samples ranging from groundwater to soil and sludge. However, we have also shown that the biosensors can detect a variety of other toxins, such as food toxins, at high sensitivity and this offers the possibility of using microbial biosensor tests as an alternative to animal testing which is currently used. This would be particularly valuable for high throughput pharmaceutical screening and is driving the effort to miniaturise the sensor format.

STUDY OF ANTIGEN/ANTIBODY INTERACTIONS THROUGH PIEZOELECTRIC MEASUREMENTS

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Immunosensors based on quartz crystal microbalance (QCM) offer attractive potentialities for mass detection. In this work, a complete and fine investigation of the QCM transducer possibilities, for realizing immunosensors, is proposed.

First of all, an ultrasensitive piezoelectric transducer, able to work in liquid with a flow through microcell, was developed. In general, resonant frequency commonly used for QCM was around 10 MHz. Here we propose a 27 MHz set up, with a good stability under a flow liquid, in order to increase the mass sensitivity : thus a direct detection without any sandwich assay, as it was in general suggested, was obtained (1,2). The quality of the measurements in term of noise and stability was achieved. Thus, due to the high quality of these measurements affinity constants are determined through a model of antibody/antigen interactions. For peroxidase/antiperoxidase couple, the affinity binding constant is compared with experimental results given by SPR measurements.

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SURFACE PLASMON RESONANCE DETECTION OF DNA HYBRIDIZATION ON POLYPYRROLE SUPPORTS

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In the field of biological analysis, the need for multiparametric tools has prompted the development of supports bearing a series of biomolecules linked to a solid support in a precise locations. In this way, we have developed a method for constructing oligonucleotide (ODN) array on gold substrate. Basically, this process involves an electrochemically directed copolymerization of pyrrole and ODN tethered to a pyrrole group leading to a one step synthesis of a solid film constituted of ODN grafted onto polypyrrole chain. This process was initially developed for the construction of Micam™ biochips bearing an array of 128 50x50µm individually addressable microelectrodes and used with a classical fluorescence detection methodology. In order to reach a label-free, real time monitoring Surface Plasmon Resonance (SPR) detection, this process has been modified to be compatible with the addressing of ODN on a non-patterned gold substrate. This new copolymerization format, namely electrospeoting, involves a 0.5 sec coupling step and the use of a pipette tip as an electrochemical cell. It allows a straightforward construction of ODN arrays by successive copolymerizations. The compatibility of these substrates with SPR imaging was checked by ODN hybridizations. Kinetic studies shows a good specificity and sensitivity; moreover, the use of a four channel sensor allows a multiparametric real time monitoring useful for the point mutation screening.

The versatility of this electrospeoting process and its miniaturization would open the use of SPR imaging to many other applications.

RECA-LUX FIBER-OPTIC BIOSENSORS TO GENOTOXICANTS

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Conservation of water resources calls for stricter regulatory measures and better monitoring systems. Whole-cell bacterial sensors have been genetically engineered by prof. Shimshon Belkin (Hebrew University) to react to target toxicants by the induction of a selected promoter and the subsequent production of bioluminescent light through a recombinant lux reporter. We have designed a one-step assay, that is self-contained, disposable and based on an optical fiber sensor module that integrates these microorganisms and a customized photodetector system. A first generation photon-counting photomultiplier tube-based instrument was constructed. Optical fiber tip cores were covered with adlayer films consisting of calcium alginate containing bioluminescent bacterial bioreporters of genotoxics. Multiplying these steps thickened the adlayers in increments, increasing the number of bacterial reporters attached to the optical fiber transducer. These whole cell optrodes are responsive to external traces of DNA damaging agents, such as mitomycin C. Light production was shown to be dose-dependent and proportional to the number of bacterial layers. All data were standardized by measuring cell counts for all tested fibers by dissolving the calcium alginate layers right after use and plating the cells on growth agar media. A number of experiments have been carried out on the optimization of the system, that include finding the right bacterial population density count in the starter probe construction and to evaluate the physiological phase which would provide us with the most efficient luminescence, including early, medium and late exponential. Results from the TECNOTOX meeting showed that our sensor was positively responsive but further improvements need to be made in order to increase higher throughput. In addition, we have synthesized a number of chemically-modified alginates in order to bring about some new properties such as the binding of biotinylated alginate beads to avidin-coated fiber optics and PEG-alginates to modulate the chemical properties of the entrapment matrix.

CONTRIBUTIONS OF ELECTROCHEMISTRY TO DNA-BIOCHIPS

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These last few years, we have developed a new methodology for the preparation of addressed DNA matrices. The **pyrrole/pyrrole-oligonucleotide electrodirected copolymerization** process is the key concept exploited by the CEA-LETI for the MICAMTM DNA-chip preparation. The resulting polymer film deposited on the addressed 50 μ m x 50 μ m gold microelectrode onto a silicon support consists of pyrrole chains bearing covalently linked oligonucleotides (see figure below). This technology was successfully applied to the genotyping of Hepatitis C Virus in blood samples. Fluorescence detection results show good sensitivity and a high degree of spatial resolution.

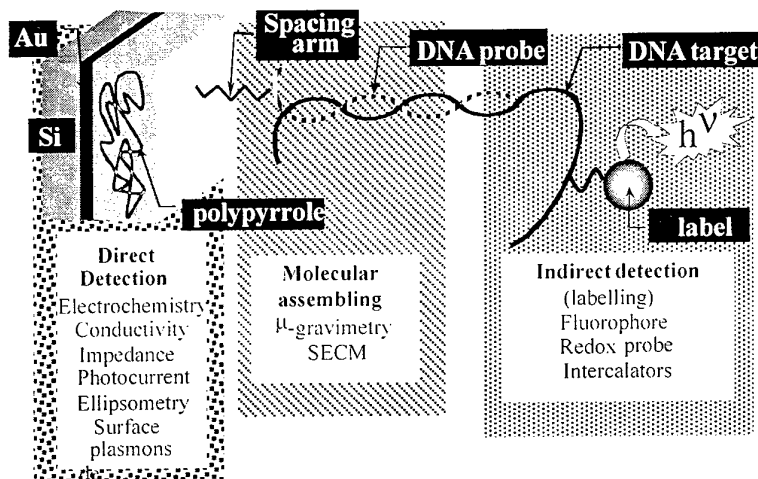


Figure :Representation of one of the 128 dots silicon/gold/polypyrrole-DNA (50 x 50 μ m²) of a MICAMTM DNA-chips, the grey tinted parts correspond to the 3 levels of transduction of the biochemical recognition event.

Electrochemistry may be exploited in other approaches of the bio-assembling:

- We have developed a "on chip" synthesis of oligo-nucleotides based on the **electrochemical deprotection** of nucleotide bases by cathodical breaking using the p-nitrobenzoyl electro-leaving group⁽¹⁾.
- **Electrodeposition of polypyrrole grafted by biotin groups** provides biotinylated surfaces with a high versatility for the immobilisation of biotin or avidin conjugates of biological entities⁽²⁾.

The development of real time, **label free** (direct detection), multiparametric detection of biological interactions are based on a change in the physico-chemical properties of the polypyrrole support during the hybridisation.

- We have recently shown that **photocurrent spectroscopy** recorded at -0.6 V/SCE allows to differentiate among the copolymer in the presence of the non-complementary and the copolymer in the presence of the complementary ODN⁽³⁾.
- **Electrospotting**⁽⁴⁾, involving a moving electrochemical cell tip has revealed to be a fruitful approach to open the **Surface Plasmon Resonance (SPR)** technique to multiparametric imaging of DNA hybridisation. The versatility of the electrospotting process and its miniaturisation would extend the use of **SPR imaging** to many other applications, particularly toward protein immobilisation.

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CONTRIBUTION OF CHEMISTS TO DNA CHIPS IMPLEMENTATION

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DNA chips are microscale devices allowing simultaneous analysis of a high amount of different DNA sequences. Such tools are based on the principle of biomolecular hybridization (specific pairing between two complementary DNA strands). Implementation of such a chip consists in grafting on well located zones of a solid support (e.g: glass or silica) known single stranded DNA sequences (e.g: oligonucleotides). These strands are used as probes to recognize their complementary sequences. Each zone ("hybridization unit") bears one type of sequence. The size of each unit may be comprised between a few 10 μ m (high density chip) to a few mm (low density chip or macroarray).

The support is covered with a saline solution containing DNA targets to detect. When complementarity between probe and target occurs, hybridization takes place on the support, indicating biological recognition. Classically, detection, identification and /or quantitation of target molecules is achieved by exhibiting zones where hybridization is effective, using targets previously labeled with either a fluorescent or radioactive moiety. This method allows global analysis of genes and their functionality. DNA chips can be used in medical research, pharmacogenomics, food industry or environment.

Implementation of our DNA chips involves combination of processes derived from chemistry and microtechnologies. One of the key point is the control of the support fonctionnalization, achieved in two steps :

- 1) Grafting of silane molecules bearing at one end a function (e.g : chlorosilane) allowing formation of a covalent bond with surface hydroxyls ; and bearing at the other end a protected chemical function. After a deprotection step, this moiety is activated thus allowing :
- 2) Either covalent immobilization of presynthesized single stranded DNA bearing a primary amine termination,
- 3) Either direct oligonucleotide synthesis on the support.

Grafted films have to be robust enough to avoid progressive damaging through following chemical processes (e.g : oligonucleotide synthesis involves aggressive reagents such as iodine, trichloroacetic acid, or ammoniac) or physical treatments (e.g : denaturation by heating or in sodium hydroxide). Ideally they should resist to several hybridization / denaturation cycles, in media containing detergents at temperature up to 95°C.

Characteristics of the chip such as reproducibility, robustness towards aggressive reagents, and generally speaking reliability, thus depend on the fonctionnalization of the support. Surface chemistry also influences the sensibility of the detection by determining the level of non specific background (adsorption of non complementary sequences onto the surface), allowing to improve the signal/noise ratio.

Other contribution of chemists to chips implementation is adaptation of oligonucleotide chemical synthesis to methods of reagents microdeposition onto the support : for instance, reagent drops spreading onto the surface depends on the used surface chemistry, oligonucleotide synthesis yield depends on the control of evaporation of these drops, choosen reagents must be compatible with materials of the microdeposition device.

We present a pannel of results obtained in the CNRS Genome Program of DNA chips implementation.

NEW APPROACHES TO OLIGONUCLEOTIDE IMMOBILIZATION FOR APPLICATION IN CHIP TECHNOLOGY

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The performance of miniaturized assays, such as biochips has improved dramatically in the past few years resulting in large-scale DNA analysis for DNA diagnostics, sequencing of genomic clones and identification of expressed genes.

The biochip can be looked upon as a sensor surface consisting of a glass slide (25 x 75 mm) coated with a thin polymer film on which biomolecules are immobilized. In order to design a surface appropriate for small molecule binding studies a functionalized polymer that provides high immobilization capacity, low non-specific binding and an excellent accessibility for the interacting molecules is required. For this purpose, silanes, polyethylenes, caoutchouc, poly(vinyl alcohol) gel pads and epoxy-polymers are used as immobilization matrix. The coupling of DNA to the polymer-coated chips is performed by use of bifunctional crosslinkers, such as adipic acid dihydrazide, glutaraldehyde and β -cyclodextrin. The reactive groups are introduced to the surface by either conventional surface chemistry or by exposure to an UV-reactive gas resulting in the insert of basic (-NH₂), acid (-SO₃H) or neutral (-CN) functional groups which can be further modified for specific immobilization of DNA.

We have immobilized unmodified and amino-modified EUB338 (5'-GCT GCC TCC CGT AGG AGT-3'), ALF1b (5'-CGT TCG (CT)TC TGA GCC AG-3'), BET42a (5'-GCC TTC CCA CTT CGT TT-3'), which hybridize to eubacterial 16S rRNA genes, on functionalized polystyrene, polyethylene, silane, poly(vinyl alcohol) and epoxy-polymer.

In order to optimize the immobilization and hybridization conditions the immobilization capacity over 16 hours, the optimum concentration and volume of spotted oligonucleotide and Cy5-labelled target, the optimum hybridization temperature and the optimum hybridization time were determined. For this purpose, three different concentrations of immobilized oligonucleotide (5, 15, and 30 pM/ μ l), three different hybridization temperatures (40, 50, and 60 °C) and times (6, 10, and 16 h) were investigated.

We mainly focus on the immobilization of EUB338, ALF1b, and BET42a on poly(vinyl alcohol)/ β -cyclodextrin gel pads. We report the effect of β -cyclodextrin concentration and pH (pH4, pH 6.8 and pH 10) on the stability and immobilization capacity of the gel and characterize the mechanical stability, the relative porosity, the swelling kinetics and the fractional water content of the PVA gels by measuring the elastic moduls, the stress-strain curve, the friction coefficient of the fluid flow through the polymet network and the ratio of the dry and fully hydrated gel.

Furthermore, we will compare our chips to commercially available chips from Surmodics, Corning, Sigma, Genescan and TeleChem.

A NEW STRATEGY FOR IN SITU SYNTHESIS OF OLIGONUCLEOTIDE ARRAYS FOR DNA CHIP TECHNOLOGY

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New and powerful molecular biology tools for biological analysis have been well developed for the investigation of diagnostic and research on gene expression, mutations and polymorphism. The main industrial actors in the field of the biological analysis have investigated different technical methods for the realization of dense array of oligonucleotides on a chip for DNA biosensor.

We show in this paper the principle and the application of a new approach to the fabrication of oligonucleotide probe arrays on a silicon substrate structured with microwells. This new strategy uses a principle of selective protection by a polymer for in situ oligonucleotides synthesis. The polymer is deposited on chosen microwells before one of the four steps of the DNA synthesis based on the phosphoramidite chemistry.

The process combines an automatic synthesizer with a micro drop dispenser for spatially localized deposition of the protective polymer. A great choice of polymer/solvent couples allows the integration of the process in the synthesis cycle of an automated synthesizer. This principle has been used to synthesize oligonucleotides in 650µm diameter microwells with different designed sequences. The length of the oligonucleotides is 20 mers and the synthesized sequences are differentiated either by one mismatch or insertion and base deletion in the center position of the oligonucleotide.

The fluorescent intensity of the biochip has been measured after hybridization with complementary fluorescent targets on an epifluorescent microscope. The results show that the discrimination of one base is achievable.

The preliminary results for the growing of oligonucleotides in a multi step fashion show the possibility of protective polymer strategy for the in situ synthesis DNA chip fabrication.

IMMOBILIZATION AND DETECTION OF DNA MOLECULES ON MICROFLUIDIC DEVICES

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Microfluidic chips have been developed which will allow the direct detection of DNA targets present in a sample. One type of chip is fabricated using a PDMS elastomer against a machined aluminum mold to produce 75 μ m channels. The channels are derivatized using carbene-generating photobiotin followed by avidin and finally biotinylated DNA probes. Fluorescently tagged complementary target DNA is then hybridized to the probe region and detected by fluorescence microscopy with a CCD.

A second type of biochip we have developed also uses biotin-avidin-biotin chemistry. We can quickly and efficiently electrodeposit biotin-LC-hydrazide onto the surface of gold, which allows a fast one step procedure to independently address gold surfaces within a microfluidic device. The subsequent attachment of avidin to the biotinylated gold surface forms the molecular sandwich necessary to further immobilize biotinylated molecules. In this work, an array of individually addressable gold band electrodes, of micron dimensions are derivatized independently with a variety of biotinylated DNA probes. Hybridization of fluorescently tagged DNA targets is again detected by fluorescence microscopy with a CCD.

CONTROLLED COMPLEX ARCHITECTURES OF BIOSENSORS BASED ON ELECTROPOLYMERIZED FILMS.

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Among basic techniques of biomolecule deposition, the immobilisation of biomolecules in electropolymerised films has been widely used for the development of biosensors. However, this physical entrapment generates steric constraints that reduce markedly the biological activity. Electropolymerisation of precursor polymers is another electrochemical method for the immobilisation of biomolecules where the reagentless attachment of proteins is performed directly at the polymer-solution interface by chemical grafting or affinity [1-5]. In comparison to the physical entrapment in polymer films, this approach preserves the catalytic activity and/or the molecular recognition properties of the immobilised biomolecules.

In this context, we report here the electropolymerisation of a chiral dicarbazole monomer functionalised with an N-hydroxysuccinimide group. The subsequent chemical functionalisation of the poly(dicarbazole) film was easily performed by successive immersions in aqueous enzyme and mediator solutions. Besides the chiral discriminatory properties of the electroactive polycarbazole films, the spatially controlled grafting of redox mediators inside the cross-linked polymer and enzyme at the polymer-solution interface allows the mediated amperometric transduction of enzyme reactions. Enhancement of stereoselective detections will be illustrated.

The electrogeneration of biotinylated films will be also reported for the simple and reproducible immobilisation of compact protein monolayers whatever the size and geometry of the electrode surface are. In addition, gravimetric measurements corroborate the successive anchoring of avidin and biotinylated enzyme monolayers. An example of bienzyme electrodes with spatially controlled distribution of the two enzymes exhibiting complementary activities, will be described.

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MOLECULAR INTERACTIONS BETWEEN ACETYLCHOLINESTERASE AND ORGANOPHOSPHORUS COMPOUNDS IN LANGMUIR AND LANGMUIR-BLODGETT FILMS

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Principal objective was to understand the molecular interactions between organophosphates and the enzymes that are inhibited by organophosphates or enzymes that catalyze the hydrolysis of organophosphates, at the air/water interface (Langmuir films) and in Langmuir-Blodgett (L-B) films and to develop a biosensor with greater sensitivity. A detailed study on acetylcholinesterase (AChE) Langmuir films and molecular interactions between AChE and OP compounds in monolayers and in Langmuir-Blodgett films was conducted. Data demonstrates that acetylcholinesterase forms a highly stable monolayer at the air/aqueous interface. Brewster angle microscopy data indicate the reversible formation of domains upon compression and decompression of the enzyme monolayer at the air/aqueous interface. The tapping mode atomic force microscopy (TMAFM) data on L-B films of AChE indicate abundant globular AChE monomers and a limited number of large and medium sized tetramer forms of AChE. The TMAFM images and UV-Vis and FTIR spectroscopic data suggest that the configuration of the enzyme was completely modified in the presence of the paraoxon and the ellipsoidal shape of AChE disappeared. As a continuation of this project, work was initiated on designing and testing of a biosensor using fluorescence labeled AChE monolayers and on characterization of Langmuir and Langmuir-Blodgett films of organophosphorus acid hydrolase and its interactions with organophosphorus compounds.

IMMOBILIZATION OF ORGANIC AND BIOMOLECULES WITH HIGH SPATIAL RESOLUTION USING THE SCANNING ELECTROCHEMICAL MICROSCOPE (SECM)

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A novel approach for micropatterning of surfaces with organic and biological microstructures using the scanning electrochemical microscope (SECM) is described. The approach is based on introducing the spatial resolution by local depositing gold particles followed by monolayer formation and functionalization. Specifically, gold patterns were deposited locally on n-Si(111) wafers with the SECM as a result of the controlled anodic dissolution of a gold microelectrode. The presence of a complexing agent, such as halide ions, provided the feedback current necessary for monitoring the microelectrode-surface distance. Deposition of microstructures, which are composed of nanocrystallites was accomplished by applying a negative potential to the silicon substrate. The deposition process has been examined in detailed and the different extrinsic and intrinsic parameters, which control the structure and distribution of the nanoparticles that are formed, have been studied. The effect of these parameters on deposition could be explained by the band structure of the silicon.

The gold patterns were further used as micro-substrates for assembling cystamine monolayers to which either fluoresceine isothiocyanate (FIT) or glucose oxidase (GOD) were covalently attached. Characterization of the organic monolayers as well as the biological activity of the enzyme patterns was carried out by fluorescence microscopy and the SECM, respectively.

Implications of this approach as well as other concepts that are currently being examined in our laboratory will be discussed.

A RAPID AND EASY PROCEDURE OF BIOSENSOR FABRICATION BY MICRO-ENCAPSULATION OF ENZYME IN HYDROPHILIC SYNTHETIC LATEX FILMS.

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Novel enzyme electrodes based on synthetic hydrophilic latex matrixes are described for the detection for glucose. Glucose oxidase was immobilised through micro-encapsulation, by the simple adsorption of enzyme-latex suspensions on the surface of a platinum electrode. Two latex films functionalised by a hydroxy or a gluconamide group were used. The response of these biosensors to glucose additions were measured by potentiostating the modified electrodes at 0.6 V / SCE in order to oxidise the hydrogen peroxide generated by the enzymatic oxidation of glucose in the presence of dioxygen. The response of such electrodes was evaluated as a function of film thickness and temperature. The sensitivity for a two layer latex based biosensor was found to be $38.78 \text{ mA M}^{-1} \text{ cm}^{-2}$ with a response time of 3-5 seconds. Moreover, a marked improvement of the thermal stability of the biosensor was observed. Only at temperatures higher than 65 °C started the enzyme to be denatured and being inactive.

AMPEROMETRIC SCREEN-PRINTED ENZYME ELECTRODES FOR THE DETECTION OF PHENOLIC COMPOUNDS.

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The application of thick-film technology to sensor fabrication has gained acceptance amongst the scientific and technical communities due to the advantages associated with the technology [1]. Immobilisation of bio-recognition molecules for biosensors and automation of the fabrication process are all possible with thick film technology. In this paper we report the immobilisation of tyrosinase within an amphiphilic polymer matrix on various screen-printed carbon electrochemical transducers (Gwent, Acheson and Dupont Carbon Inks). The apparent surface concentrations of amphiphilic polymers for each electrode was determined to be in the order of Gwent>Acheson>Dupont. This trend can be explained in terms of the electroactive surface area of each printed electrode. As a consequence of this physical property, the amount of enzyme molecules entrapped, which was estimated by colorimetric methods, also followed a similar trend (Gwent>Acheson>Dupont). Moreover, the presence of the amphiphilic polymer on the surface of the electrode dramatically altered the voltammetric behaviour of a redox analyte compared to an unmodified electrode. All fabricated bio-electrodes exhibited an increase in peak potential separation, due to an increase in charge-transfer resistance, with an increase in peak anodic/cathodic currents. The latter observation can be attributed to the ion-exchange properties of the film. When the analytical property of the fabricated bio-electrodes was assessed with the substrate catechol Gwent electrodes exhibited a greater sensitivity for the analyte compared to Acheson and Dupont electrodes. Furthermore, Gwent electrodes displayed sensitivities 3.75 times higher than sensitivities reported for similar tyrosinase-amphiphilic modified electrodes [2]. However, the linear range for Gwent electrodes (0.025-14 μM) was considerably smaller than for Acheson (0.025-41 μM) and Dupont (0.025-33 μM) electrodes. This is explained in terms of the permeability properties of the film and film density.

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DESIGN OF DNA CHIPS: THEORETICAL ISSUES

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Two aspects of the design of DNA chips pose well defined theory problems. The first concerns the shift in the "melting temperature" of grafted DNA in comparison to free DNA. The function of DNA chips depends on the higher melting temperature of the grafted oligo nucleotides. The second concerns strategies for the repression of protein adsorption. This talk will address these two issues, in particular: (i) the factors that determine the melting temperature of grafted helicogenic chains and (ii) The design of PEO brushes that repress protein adsorption.

ELECTROCHEMILUMINESCENCE AS A TOOL FOR THE DEVELOPMENT OF ELECTRO-OPTICAL BIOSENSORS

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Several optical biosensors, based on the catalyzed chemiluminescence reaction of luminol, have been described for the measurement of hydrogen peroxide or metabolites [1]. Another way to produce light from luminol and hydrogen peroxide is electrogenerated chemiluminescence [2, 3]. In this process, luminol is electrochemically oxidized using a positively biased electrode and, in the presence of hydrogen peroxide, the light emission occurs. This principle has been exploited to develop fiberoptic sensors involving specific H_2O_2 -generating oxidases immobilized on various supports for the flow injection analysis of lactate, glucose, cholesterol and choline.

In a first approach, the electrochemiluminescence of luminol was triggered using a glassy carbon electrode (GCE) polarized at +425 mV vs. a platinum pseudo-reference electrode. The enzymatic sensing layer was in close contact with the GCE and faced a liquid core single optical fiber, the other end of which was connected to the photomultiplier tube of a luminometer. After optimization of the operating and reaction conditions, the following detection limits ($S/N = 3$) were obtained: 30 pmol, 60 pmol, 0.6 nmol and 10 pmol for lactate, glucose, cholesterol and choline, respectively [4 - 6].

Afterwards, miniaturization of electrochemiluminescence-based sensors was achieved using screen-printed electrodes instead of glassy carbon macroelectrodes. To demonstrate the feasibility of this approach, choline oxidase was used as a model H_2O_2 -generating oxidase [7].

Based on this electrochemically-induced luminescence technique, a fiberoptic immunosensor for the detection of the herbicide: 2,4-dichlorophenoxyacetic acid (2,4-D) was also described. In that case, luminol was used as an electroactive label and the detection limit was the same (0.2 $\mu g/l$) than that obtained with peroxidase-labeled antibodies [8].

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THE MICROSCALE APPLICATION OF THE FLUOROMETRIC ANALYSIS OF DNA UNWINDING (μ -FADU) AS A MAMMALIAN GENOTOXICITY ASSAY

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The fluorometric analysis of DNA unwinding (FADU assay) was originally designed for rapid detection of X-ray-induced DNA damage in mammalian cells. This cellular bioassay is based on time-dependent alkaline denaturation of DNA under moderate denaturing conditions (pH 12.2-12.4) starting from ends as well as from all DNA break points (single-strand breaks, DSB; double-strand breaks, DSB; alkali-labile sites, ALS). DNA which remained double-stranded after 30 min of alkaline treatment was detected after neutralisation and immediate fragmentation by binding to the Hoechst 33258 dye (bisbenzimidazole) and measuring its fluorescence. The current paper describes a modification of the method by use of a microplate fluorescence reader which allows cell cultivation, chemical treatment and analysis of 96 samples in the same plate. Three different treatment approaches were performed: (i) treatment of cells for 60 min at ice temperature allowing identification of direct acting substances capable of DNA strand break induction, (ii) treatment of cells for 60 min at 37°C in absence or presence of the DNA polymerase inhibitor aphidicolin allowing identification of direct acting substances which induce strand breaks either directly or during repair of genotoxic damages and (iii) treatment of cells for 60 min at 37°C in absence or presence of the DNA polymerase inhibitor aphidicolin and with S9 metabolic activation allowing identification of the action of indirect acting substances. For the verification of the μ -FADU method typical model compounds with various DNA damaging potencies were tested. The results demonstrate, that the μ -FADU approach is suitable to distinguish between the different DNA lesions (strand breaks versus base alterations) preferentially induced by different environmental radiations (X-rays versus UV) and to distinguish between the different biochemical processes during damage repair (incision versus polymerisation and ligation).

DESIGN OF MOLECULAR ASSEMBLIES OF P450 ENZYMES FOR NANOBIO TECHNOLOGY

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The cytochrome P450 enzyme family is responsible for the metabolism of a large number of drugs and xenobiotics. These enzymes are highly relevant to human and animal health as they play a key role in the pharmacodynamics of drugs. Despite their importance, structural studies are impaired by problems related to their poor interaction with electrode surfaces and their association to biological membranes. Very recently a soluble, bacterial P450 enzyme (P450 BM3) has been found to share a high degree of homology with the human systems.

An exciting potential application of these enzymes relies in the creation of electrode arrays for high-through-put screening for propensity to metabolic conversion or toxicity of novel potential drugs. In order to achieve this goal, three issues need to be addressed: 1. the efficient electron transfer of the P450 with the electrode surface, 2. the ability of handling stable and soluble human P450 enzymes, and 3. the availability of a screening method able to identify active variants of P450 to be used to produce arrays with different properties. The first two issues have been addressed with the *molecular lego* approach. Mimicking the natural molecular evolution that proceeds at DNA level by modular assembly of introns and exons, flavodoxin from *D. vulgaris* and the soluble haem domain of cytochrome P450 from *B. megaterium* have been used to produce an efficient, artificial electron transfer chain. The scaffold of this soluble enzyme have been used to build in the key structural and functional elements of the human cytochrome P450 2E1. The chimeric protein containing the reductase and P450 domain has been successfully constructed and expressed.

ENHANCEMENT OF THE IONIC RESPONSE OF FIELD EFFECT TRANSDUCERS USING POROUS SILICON

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This presentation shows the features of the oxidised porous silicon /silicon structures functionalized with calixarene molecules for the ionic detection.

The sensitivities to sodium ions of flat silica/silicon structures and oxidised porous silicon/silicon structures both functionalized with a deposited thin film of p-tert-butylcalix[4]arene molecules are compared. The variation of the flat-band potential of the functionalized flat silica/silicon structures versus concentration of sodium ions is about 56mV/pNa. When the porosity of the oxidised porous silicon varies from 57% to 65%, the sensitivity of the functionalized oxidised porous Si/Si varies from 180 mV/pNa to 233 mV/pNa. these are very large values of sensitivities when compared to the Nernstian one. In order to explain such an over-Nernstian response, we have studied the effect of the porous layer parameters (porosity, pore morphology, porous layer thickness, and the internal surface area) on the sensitivity.

A physical modelization of the formation of the depletion layer in the oxidised porous silicon substrate, is proposed through a tunnelling effect model due to the quantum-sized clusters within the porous layer. The effect of the pore size on the sensitivity of the functionalized oxidised porous Si/Si structures is also studied in order to validate the model.

A second calixarene molecule, which is known as a metallic copper ion detector, has been deposited on oxidised porous silicon/silicon samples in order to study the effect of the ion valence on the sensor response, and thus to generalise the electrochemical equation, which governs the response of such field effect structures. Actually, we have recorded values of potentiometric sensitivities between ~ 70 mV/pCu and ~ 100 mV/pCu for a porosity range from 57% up to 65%. We have also studied the effect of the temperature on the properties of these ionic sensors and we have calibrated their thermal sensitivities versus the porous layer characteristics.

SINGLE MOLECULE STUDY OF DNA-FORMAMIDE INTERACTION

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We present a study of the reaction kinetics between double stranded DNA (dsDNA) and formamide, HCONH_2 , in a single DNA molecule. Our single molecule studies are based on the coverslip-DNA-bead construct and on an Optical Tweezer system. λ -DNA molecules (Promega, 48.5 Kb, contour length $16.5\text{ }\mu\text{m}$) are attached to polystyrene microbeads ($2.8\text{ }\mu\text{m}$, Polysciences) at one end and to the glass coverslip at the other using a low pH protocol. The bead is trapped by the Optical Tweezer allowing to both manipulate and visualize the corresponding end of the DNA. Stretching the DNA to its maximal length with predetermined force (e.g. 8 pN) one can deduce its contour length.

In this setup we monitor changes in the contour length of the DNA that result from chemical reactions occurring on the DNA. In particular, formamide is a denaturing agent whose NH_2 group is replacing the DNA bases in the inter-strand hydrogen bonds. Since its volume is larger than that of a hydrogen bond, its side effect is to slightly enlarge the distance between adjacent bases. Therefore, a certain concentration of formamide on the DNA is equivalent to a particular change in its contour length. We monitor the kinetics of the reaction by measuring the length of the DNA at 1 minute intervals. We also study the changes in the kinetics resulting from applying a fixed force to the DNA. We find that applied force: i. speeds up the reaction and ii. modifies the final steady state.

POSTERS

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P1

NEURONAL NETWORK ACTIVITY: SENSITIVE BIOSENSOR FOR THE PRESENCE OF ENVIRONMENTAL POLLUTANTS.

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The central nervous system (CNS) is a major target affected by variety of toxic compounds and metals. Neurons, by highly specialized intrinsic electrophysiological mechanisms, represent transducers that report cell conditions: Single neurons and circuit activity introduce distinctive patterns of action potential (AP) activity, however, these patterns are highly sensitive to the presence of drugs, metals and different compounds in their extra-cellular environment. Neuronal cells can be maintained over weeks in culture conditions, expressing simplified phenotype of the parent tissue. The cells develop networks of neurons that communicate with each other in a characteristic dynamic spatio-temporal pattern. Neuronal networks cultured on microelectrode array are suitable for continuous monitoring of electrical activity along time and therefore they may serve as high sensitivity sensor.

Here, we apply these technologies for detection of short and long-term consequences of exposure to GABA, Baclofen and Muscimol. In order to define the action of these compounds we developed a set of tools that enable the characterization of native pattern of activity and define a significant discrepancy. AP frequency, inter-spike-interval, minimal inter-spike-interval, auto and cross correlation function, burst frequency and duration were examined for determination of distinctive patterns of activity.

Short exposure of neuronal network to GABA induce short period of unsynchronized AP firing, which changed within 24h to highly synchronized AP burst, both changes in the neurons and network pattern of activity can be detected by measure of minimal inter-spike-interval, auto and cross correlation function. AP frequency and burst duration were not distinctive for changes in the native activity. We suggest the use of neuronal network as a sensitive biosensor for monitoring and detection of environmental pollutants.

P2

INCLUSION OF METAL MICRO-PARTICLES INTO POLY(PYRROLEALKYLAMMONIUM) FILMS CONTAINING GLUCOSE OXYDASE FOR THE AMPEROMETRIC DETECTION OF GLUCOSE.

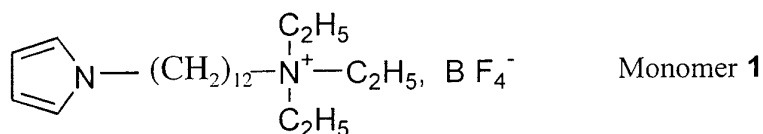
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The major problems facing glucose biosensors deal with the operational stability of the biological element and the elimination of interfering species. In this contribution, we have designed a route of biosensors improvement based on the inclusion of metal particles in the proximity of immobilised enzyme glucose oxidase (GOD). In such a way, the hydrogen peroxide generated by GOD metabolism may be oxidised in the vicinity of the enzyme thus reducing its deactivation. Moreover, by controlling the nature of the metal micro-particles, it becomes possible to design amperometric biosensors exhibiting a more appropriate working potential.

Glucose biosensors were fabricated through the immobilisation of GOD onto *in situ* electrogenerated polypyrrole films. The enzyme entrapment was managed by an original technique which involves the electropolymerisation of a previously adsorbed layer of GOD and of amphiphilic pyrrolalkylammonium monomer **1**.



The aforementioned method was applied to the generation of biolayer containing GOD onto electrode surfaces of platinum or carbon. Owing to the anionic exchange properties of the obtained polypyrrole films, we have incorporated metal micro-particles within the biolayer, thus extended the transduction surface to the volume of the biofilm, by electrochemical reduction of electrostatically incorporated metal salts (such as hexacyanoplatinate for example).

This contribution demonstrate the feasibility of the process through the inclusion of platinum micro-particles into polypyrrole-GOD layers generated onto carbon electrodes. Platinum inclusions allows to decrease significantly the working potential of the obtained biosensor. The operational stability and the response to model interfering species of the biosensor were tested and compared to glucose sensor based on the immobilisation of GOD onto platinum electrodes.

P3

SCREEN-PRINTED ELECTROCHEMICAL BIOSENSOR IN A MICRO-FLOW INJECTION SYSTEM FOR THE DETECTION OF THE ENVIRONMENTAL POLLUTANTS.

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An amperometric micro-flow injection biosensor for the identification and quantification of environmental pollutants such as phenol and organophosphorus compounds (DDVP) was developed. The electrochemical cell consists of screen-printed electrodes covered with an enzymatic membrane and placed in a micro-flow injection system that allows to significantly reduce the volume of the detected samples down to 5 μ l.

Phenol detection occurred with the enzyme tyrosinase, which oxidized phenol into catechol, then itself into quinone, its reduction by the electrode generating a measurable cathodic current.

The biosensor for the detection of organophosphorus compounds consisted in the inhibition of acetylcholine esterase via the decrease in enzymatic degradation of the substrate, acetylthiocholine chloride, to thiocholine and acetate. Thiocholine reacts with the mediator Os(bpy)₂-polyvinylpyridine, which is immobilized on the working electrode. Its subsequent reoxidation by the electrode generates electric signals.

The immobilized electrode was electrochemically characterized by cyclic voltammetry and the enzymatic and electrochemical reactions were then examined and optimized.

We also investigated the dependence of the response of the enzyme electrode on the flow rate, the amount of loaded enzyme, the pH, as well as the type of immobilization utilized. The detection limit for phenol and DDVP were 10ng/ml and 0.1ng/ml respectively.

The sensor is of low cost, easy to operate and was shown to meet the sensitivity requirements of the European Community.

P4

APPLYING BACTERIAL LUX-FLUORO-TEST AS AN ALTERNATIVE METHOD FOR ASSESSING OCULAR TOXICITY OF WATER-SOLUBLE AGENTS

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The SOS-Lux-Fluoro-Test is a reliable bioassay for fast detection of environmental pollutants, which coincidentally measures the cytotoxic and genotoxic potency of a given substance based on the receptor reporter principle. In this bioassay, a strong SOS promoter is applied as receptor component for DNA damaging effects and mediates induction of the promoterless *luxCDABFE* genes of *Photobacterium leiognathi* which represent the bioluminescent reporter component.

When testing cytotoxic agents, quantification of SOS system induction may be influenced by dying bacterial cells. Subsequently it is reasonable that viability of the bacteria is included as a test parameter describing the cytotoxicity of the agents. For that reason *Salmonella typhimurium* TA1535 cells were transformed with the plasmid pGFPuv, in which the green fluorescent protein expression is controlled by the constitutive lac promoter.

Recombinant *S. typhimurium* strains carrying either the SOS-Lux plasmid (TA1535-pPLS-1) or the fluorescence mediating lac-GFPuv plasmid (TA1535-pGFPuv) were used to determine simultaneously genotoxic and cytotoxic responses of several water-soluble agents which are known to produce a broad range of Draize rabbit eye irritation test maximum average scores. These data were related to the responses of control substances which have different known potentials. In vitro data, averaged from replicate assays were comparable to respective Draize rabbit eye irritation data from the publicly available ECETOC database. Due to this strong correlation and practical advantages, especially when using a microplate reader, this assay system might be applied for prescreening of pharmaceutically relevant drugs as well as a useful tool for animal test reduction.

P5

FIBER OPTIC REC A-LUX SENSORS TO GENOTOXICANTS: SYSTEM OPTIMIZATION

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An *Escherichia coli* strain, genetically modified to emit a luminescent signal in the presence of genotoxic agents, was alginate-immobilized onto an exposed core of a fiber optic. The performance of this whole-cell optical fiber sensor system was examined as a function of several parameters, including gel probe matrix volume, bacterial cell density, numerical aperture of the fiber core and working temperature. An optimal response to a model genotoxicant, mitomycin C, was achieved with 6 alginate/bacterial adlayers on a 1 cm exposed fiber optic core. Total alginate volume per tip was about 100 μ l, containing a bacterial suspension of around 1.5 to 3.0×10^7 cells. When the core diameter was etched down to 270 μ m from 400 μ m to optimize the numerical aperture, photon detection efficiency significantly increased, although to a lesser extent than that expected from theoretical calculations. Further reduction in core diameter led to a reduced performance. Activity at 37 °C was superior to that at 26 °C. Under these optimized conditions, the optrode biosensor response for mitomycin C was shown to be dose-dependent for at least 6 hours, with a lower detection threshold of 25 μ g/l.

P6

FIBER OPTIC REC A-LUX SENSORS TO GENOTOXICANTS: STORAGE BEHAVIOR

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Environmental monitoring systems have been produced that utilize whole-cell bacterial bioreporter sensors (genetically engineered to react to target toxicants by the induction of a selected promoter while producing bioluminescent light through a recombinant *lux* reporter). We have designated a one-step assay that is self-contained, disposable and based on an optical fiber sensor module that integrates these microorganisms and customized photodetector system. Optical fiber tip cores were covered with adlayer films consisting of calcium alginate containing bioluminescent bioreporter. These whole cell optrodes were responsive to external traces of DNA damaging agents, such as mitomycin C or N-Methyl-N'-nitro-N-nitrosoguanidine. Light production was shown to be dose-dependent and proportional to the number of bacterial adlayers. Storage issues have been addressed, such as the long-term storage of the optrodes. In order to carry out these studies, microtiter assays were undertaken first with alginate beads containing the bioreporter cells. Results indicate that the optrodes retain activity over storage periods of two weeks at least, while long-term storage leads to loss of response sensitivity.

P7

A COLORIMETRIC BIOSENSOR FOR THE SCREENING AND DETECTION OF MEMBRANE PENETRATION ENHANCERS

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Membrane penetration enhancers are essential compounds for altering skin barrier properties to percutaneous absorption of compounds, especially at the level of the stratum corneum, allowing a drug to reach the living tissues at a greater rate. In this frame, we have developed novel colorimetric biosensors, composed of phospholipids embedded in a matrix of polymerized diacetylene [PDA], which can be used for screening and detection of membrane permeation and disruption by penetration enhancers. The activities of the monounsaturated fatty oleic acid, Azone (1-dodecylazacyclohepta-2-one or laurocapram), propylene glycol, polysorbate and transcutool (ethoxydiglycol) have been evaluated with the colorimetric biosensors. UV-vis spectroscopy, steady-state fluorescence anisotropy, transmission electronic microscopy, and dynamic light scattering have been used to study the colorimetric systems. The blue-to-red transition of these biosensors is highly correlated with the disorganization of stratum corneum lipids.

P8

IMMOBILIZATION AND DETECTION OF DNA MOLECULES ON MICROFLUIDIC DEVICES

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Microfluidic chips have been developed which will allow the direct detection of DNA targets present in a sample. One type of chip is fabricated using a PDMS elastomer against a machined aluminum mold to produce 75 μm channels. The channels are derivatized using carbene-generating photobiotin followed by avidin and finally biotinylated DNA probes. Fluorescently tagged complementary target DNA is then hybridized to the probe region and detected by fluorescence microscopy with a CCD.

A second type of biochip we have developed also uses biotin-avidin-biotin chemistry. We can quickly and efficiently electrodeposit biotin-LC-hydrazide onto the surface of gold, which allows a fast one step procedure to independently address gold surfaces within a microfluidic device. The subsequent attachment of avidin to the biotinylated gold surface forms the molecular sandwich necessary to further immobilize biotinylated molecules. In this work, an array of individually addressable gold band electrodes, of micron dimensions are derivatized independently with a variety of biotinylated DNA probes. Hybridization of fluorescently tagged DNA targets is again detected by fluorescence microscopy with a CCD.

P9

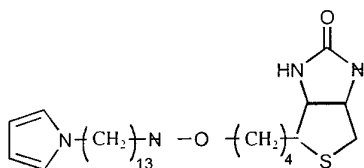
EVALUATION OF NON-SPECIFIC RECOGNITION OF AVIDIN ONTO ELECTRONIC CONDUCTING POLYMERS

Dupont Agnès, Billon Martial, Bidan Gérard

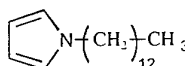
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We report here an original approach for the insertion of biochemical entities in Electronic Conducting Polymers (ECP) film via the synthesis of a biotinylated polypyrrole (1,2). Because of the biotin-avidin affinity, biochemical entities bearing avidin units can bind to the biotin units grafted on the polypyrrole network. The biotinylated polypyrrole described here is a homopolymer in which the biotin is linked to the nitrogen atom of a pyrrole unit by a hydrophobic spacer arm (monomer a). We have chosen a spacer arm sufficiently long to ensure unrestricted recognition of biotin by avidin and hydrophobic to studied the obtained biotinylated polypyrrole (ppy) films in non-aqueous media.

To investigate the affinity of the biotinylated ppy coatings versus avidin, we have carried out gravimetric measurements using Quartz Crystal Microbalance (QCM). The polypyrrole-biotin was coated onto quartz surface by electropolymerization of modified pyrrole monomers in solution. In presence of avidin, a rapid decrease of the microbalance resonance frequency was observed. This frequency shift was attributed to immobilization of avidin onto the biotinylated film by both specific binding of avidin/biotin and non-specific binding due to adsorption of avidin onto the film. To evaluate this event, the same experiment was performed on a ppy film electrogenerated from the same monomer precursor which does not contain the biotin group (monomer b).



monomer a



monomer b

We have investigated the electrochemical behaviour of the two modified monomers and their related polymers. The QCM results demonstrated the occurrence of unspecific adsorption of avidin onto the non-biotinylated film which was influenced by several experimental parameters (e.g. film thickness, electronic state of polymer...). We have verified that the amount of absorbed avidin was dependent on the film thickness and also on the potential applied to the film. The optimum thickness and applied voltage have been determined with the aim to limit the non-specific adsorption of avidin. Under these conditions, we have evaluated the specific binding of avidin/biotin more accurately.

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P10

AMPEROMETRIC GLUCOSE BIOSENSOR BASED ON ENTRAPMENT OF GLUCOSE OXIDASE DURING ELECTROCHEMICAL POLYMERIZATION OF POLY(3,4-ETHYLENEDIOXYTHIOPHENE)

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Entrapment of redox enzymes in growing conducting polymers has potential applications in the preparation of amperometric biosensor. A glucose sensor was obtained from glucose oxidase (GOD) entrapped within a polymer film during electrochemical polymerization of 3,4-ethylene-dioxythiophene (EDT).

The potential of the platinum electrodes was scanned from + 0.2 V to + 1.5 V (vs. SCE) at a rate of 100 mV/s, in phosphate buffer solution (20mM, pH 6.2). Glucose was detected at 0.65 V (vs. SCE) under oxygenated condition.

The influence of various parameters related to the electropolymerization process was explored to optimize the analytical performance of the biosensor.

The response curve is approximately linear up to 8mM and a detection limit of 4×10^{-5} M was found. Two to five seconds was needed to reach 95% steady state response. Stability of electropolymerized films was evaluated in storage and operational conditions. The glucose probe stored in buffer at 4°C when not in use, showed a residual activity of about 40 % after 3 weeks.

Keywords

Conducting Polymer films, PEDT, Enzyme electrodes, Voltammetry, Glucose

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P11

ELECTROPOLYMERISABLE PYRROLE-OLIGONUCLEOTIDE : SYNTHESIS AND ANALYSIS OF ODN HYBRIDIZATION BY FLUORESCENCE AND QCM

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Pyrrole has been extensively used for electrode surface modification by biological molecules owing to its ability to electrochemically polymerise in aqueous media. Moreover, compared to other techniques such as self assembled monolayers or sol-gel for example, pyrrole enables to address specifically either microelectrodes or macro substrates.

In such a way, we have designed a new route for electrodes (or conductive surfaces) modification by ODN probes for hybridization-based DNA recognition. The process includes the electrochemical copolymerisation of pyrrole and oligonucleotides bearing on their 5' end a pyrrole moiety introduced by phosphoramidite chemistry. The electro-controlled synthesis of the copolymer gives in one step an homogenous polypyrrole film deposited on the electrode surface without deactivation of the recognition abilities of the ODN probes.

The presence, the repartition homogeneity and the activity of the immobilised ODN probes were revealed by fluorescence spectroscopy owing to fluorescent probes coupled to the hybridised strands by biotin-streptavidin interaction. Fluorescence has highlighted the specificity of the built up biolayers and the reliability of the immobilisation step.

Fluorescence enables the end point detection of DNA hybridisation excluding kinetic recording of the recognition phenomena. In this aim quartz crystal microbalance appears as a well suited technique for the real time monitoring of DNA hybridisation. After quartz modification by an ODN-containing copolymer, we have recorded the mass uptake of the biolayer consecutive to the recognition event. The latter was further amplified by coupling streptavidin to the hybridised strands thus demonstrating the reliability of QCM transduction.

P12

HYDROGEN ENZYME ELECTRODES BASED ON *TH. ROSEOPERSICINA* HYDROGENASE IMMOBILIZED ON DIFFERENT CARBON SUPPORTS

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Hydrogenase is unique enzyme able to catalyze proton reduction and hydrogen oxidation. Hydrogen enzyme electrode is the system in which these reactions are coupling with electron exchange between enzyme and electrode. It was first developed using the enzyme from *Thiocapsa roseopersicina* [1] adsorbed on carbon black electrodes in 1984.

The hydrogenase was immobilized by its adsorption onto two carbon electrodes made from fibrous material (type TVS, NPO «Graphite», Moscow) and graphite (Ringsdorf, Germany). The pre-treatment of the surface and the porosity of support affect the bioelectrocatalytic activity of enzyme electrodes. The influence of neomycin as a promoter for orientation of hydrogenase molecule during immobilization have been studied.

The equilibrium hydrogen potential on inert carbon support with immobilized hydrogenase was achieved. It seemed that the electrochemical stages of electron transfer between the enzyme active sites and the electrode were the limiting ones. Indeed, the steady-state current-voltage curves were fit to the two-exponential equation evaluated for the two sequential one-electron electrochemical stages.

The hydrogenase electrodes have demonstrated the high stability during the storage. The carbon fibrous electrode has lost about 10% of initial activity after keeping in refrigerator for a month. Even after 6 months of storage the electrode has remained up to 50 % of the initial hydrogenase activity. Soluble enzyme samples in similar conditions have lost their activity after 10 days.

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P13

BIENZYME ELECTRODES (POLYPHENOL OXIDASE - ALKALINE PHOSPHATASE) : ELABORATION AND CHARACTERIZATION OF SPACIALLY CONTROLLED ASSEMBLIES.

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An innovative electrochemical method of biomolecule immobilization consists in the electrogeneration of electronically conducting polymers functionalized by biotin groups. Owing to the remarkable high affinity of avidin for biotin coupled enzyme, the attachment of biotinylated enzymes on biotinylated polypyrrole films has been reported to be a simple and reproducible immobilization process of compact protein monolayers whatever the size and the geometry of the surface [1-3].

In the present work, we report the step by step construction of a bienzyme electrode (polyphenol oxidase - alkaline phosphatase) with a spatially controlled distribution of two enzymes exhibiting complementary activities. The soft anchoring of the biotinylated polyphenol oxidase (PPO-B) and avidin labelled alkaline phosphatase (AP-A) was accomplished by bioaffinity layering based on the growth of alternate monolayers by avidin-biotin bridges on an underlying electropolymerized biotin polypyrrole film. Gravimetric measurements were carried out using quartz crystal microbalance (QCM) to characterize the efficient coupling of avidin, PPO-B and finally AP-A to the biotinylated polymer film. The analytical characteristics of monoenzyme electrodes (PPO-B electrode and AP-A electrode) was first investigated for the amperometric detection of catechol, phenol and phenyl phosphate, respectively. The performance of the bienzyme electrode has been then examined for measurements of phenyl phosphate. Special attention has been paid on the design of the enzyme architecture in comparison to an other immobilization process which consists to the entrapment of the two enzymes within an amphiphilic ammonium polypyrrole matrice [4].

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P14

A GLUCOSE BIOSENSOR BASED ON MODIFIED-ENZYME INCORPORATED WITHIN ELECTROPOLYMERIZED POLY(3,4-ETHYLENEDIOXYTHIOPHENE) (PEDT) FILMS

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We have constructed and characterised a glucose sensor using glucose oxidase (GOD) covalently attached to carboxylic acid polyethyleneglycol (PEG), called (PEG-GOD). The latter modified enzyme was entrapped afterwards within poly(3,4-ethylenedioxythiophene) (PEDT) films electrogenerated on glassy carbon (GC) electrodes. The composite (PEG-GOD/PEDT) film is more porous than the film without enzyme (PEDT+PEG). Data from EQCM (Electrochemical Quartz Crystal Microbalance) and pH-stat experiments indicate a good relative activity of the modified enzyme, ca. 10%. Amperometric measurements, using ferrocenemethanol as redox mediator, confirms that the modified enzyme is catalytically active. The effect of film thickness was also investigated. The sensitivities were quite similar for modified-GOD electrodes (ca $3 \text{ mA cm}^{-2} \text{ M}^{-1}$) and unmodified-GOD electrodes (ca $2.6 \text{ mA cm}^{-2} \text{ M}^{-1}$) but a better stability was obtained with modified PEG-GOD electrodes.

P15

A CHEMILUMINESCENT OPTICAL FIBER IMMUNOSENSOR TO MONITOR *LISTERIA MONOCYTOGENES*

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The development of a diagnostic test kit, based on biosensor technology, to monitor the presence of an important food pathogen, *Listeria monocytogenes*, is proposed herein. Persistent listeriosis outbreaks continue to plague industrialized countries. Food industries have lost hundreds of millions of dollars due to *Listeria monocytogenes* contamination in food. To help reduce these losses, rapid diagnostics capable of pin-pointing the presence of the aforementioned etiological agent are sought. Conventional assays rely on classical growth plating techniques alone or in combination with state-of-the-art DNA hybridization or genetic PCR amplification techniques. Whichever method is used, a minimum of 48 hours (and up to 5 days) are required to obtain the results. This time constraint is a serious economical handicap to food producers. There is therefore a need for a practical, sensitive and rapid diagnostic method that would detect with a high degree probability the presence of *Listeria monocytogenes*.

A novel optical fiber biosensor is proposed. Optical fiber transducers specifically chemically modified to conjugate an immobilized molecular recognition element, such as outer-wall anti-*Listeria monocytogenes* immunoglobulins. The antibodies will then capture the target bacterial cells in the enrichment broth. Thereafter, the glass fiber is incubated with a second anti-*Listeria monocytogenes* immunoglobulin labelled with the horseradish peroxidase. Chemiluminescence is generated. This light signal is then transduced through the core of the optical fiber and is immediately transformed into measurable electric signal wherefrom the end-user obtains a printed results.

P16

RED AND ENHANCED GREEN FLUORESCENT PROTEIN (DSRED / EGFP) IN TWO-PARAMETER BIOASSAYS

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Fast bioassays for toxic effects in mammals require the use of appropriate reporters in a mammalian cell system. Fluorescent Proteins (FP) of different colours offer the possibility to study kinetic processes in living cells. Recently, the availability of Red Fluorescent Protein (DsRed) from *Discosoma sp.* expanded the spectrum of FP.

The suitability of separate and combined measurement of EGFP and DsRed was examined in order to create a two-parameter bioassay for gene expression and cytotoxicity.

EGFP and DsRed (Clontech) were transfected into Chinese Hamster Ovary (CHO) cells. In transient and stable transfections, strong DsRed and EGFP expression in CHO cells was observed in the fluorescence microscope (excitation BP 546/12, emission LP 590; excitation BP 450-490, emission LP 520, respectively).

Multiplying EGFP and DsRed fluorescence spectra by filter transmission data per wavelength allowed estimation of the fraction of fluorescence recorded. In the microplate reader, the filter set 540/25+590/35 allows higher yields of DsRed fluorescence than 540/25+620/40, while for EGFP, the filter combination 460/40+508/20 yields higher readings than 485/20+530/25. These filter pairs allow an optimal differentiation between EGFP and DsRed.

In FACS-analysis, the argon laser (488 nm) meets a minor peak of the DsRed excitation spectrum and allows a suboptimal measurement of fluorescence in FL-2. In mixed populations of unmodified and DsRed or EGFP-expressing cells, the three populations can be identified by their green and red fluorescence.

The two examined reporter proteins EGFP and DsRed are suitable for construction of a two-parameter-bioassay based on microplate reader measurements or FACS analysis.

P17

DEVELOPMENT OF AN INTEGRATED MICROSENSOR ARRAY FOR REMOTE ENVIRONMENTAL MONITORING

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The environmental management of our freshwater ecosystems is an increasingly important issue. Therefore an integrated microsensor array, which would continuously monitor the general health of these environments, would be an extremely useful analytical tool.

Dissolved oxygen, temperature, pH and water hardness are all markers of freshwater quality that may collectively indicate any variation in local environmental health. The design of an integrated microsensor array to measure these parameters has been considered. A modular approach to system design has been taken, so as to limit the complexities of microfabrication. Interconnection of individual microsensors and controlling electronic circuitry has been considered with a generic "interface-substrate" being incorporated into the overall system design, along with a simple flow channel and a novel, electrokinetic, anti-fouling technique. The latter is incorporated to prevent the formation of a biofilm on the microsensor surfaces, which would seriously degrade device performance. Computer simulations of this electrochemical technique have been performed.

A combined dissolved-oxygen and temperature microsensor has been developed, where a thin-film nickel thermal resistor is employed as a temperature sensor and dissolved-oxygen is measured amperometrically by a parallel array of microelectrodes and a Ag|AgCl reference-counter electrode. A significant increase in current density was observed as the diameter of individual microelectrodes in the cathode array was decreased and their number proportionally increased. The enhanced mass transport properties of the smaller microelectrodes give rise to an increase in current density and the problems associated with the minute currents of such electrodes are overcome by employing a parallel array.

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